ENDOCRINOLOGY

VOLUME 33 AUGUST, 1943 NUMBER 2

NATURE OF THE PITUITARY FACTOR STIMULATING MAMMARY DUCT GROWTH[1]

J. J. TRENTIN, A. A. LEWIS, A. J. BERGMAN AND C. W. TURNER

From the Department of Dairy Husbandry, University of Missouri
COLUMBIA, MISSOURI

THAT the pituitary contains some factor or factors involved in mammary growth is generally accepted. However, as to the exact nature, number and mode of action of such entities there has been debate (Greep and Stavely, 1941; White 1943). The present paper deals with the question of the chemical nature (*i.e.* protein or lipid) of the pituitary factor responsible for the stimulation of mammary duct growth in the male mouse.

Using the male mouse in an assay technique, Lewis and Turner (1938) reported that the drying and defatting of fresh pregnant cattle pituitary with acetone and ether resulted in a 60 per cent loss of mammary duct-growth stimulating activity (mammogen I) present in the fresh gland. However, the extraction of fresh tissue with 60 per cent alcohol, followed by precipitation of lactogenic and other protein hormones by adjusting the pH to 5.7 and increasing the alcohol content to 86 per cent, yielded, upon vacuum distillation, an oily residue containing 90 per cent of the activity of the fresh tissue. It was later reported that upon ether extraction of this physiologically active preparation the ether-soluble fraction retained activity while the ether-insoluble fraction was inactive (Lewis and Turner, 1939). Extraction of fresh pituitary with hot ether-alcohol (1:3) appeared to recover 100 per cent of the potency.

Mixner, Lewis and Turner (1940) using the ovariectomized virgin female mouse assay for the mammogenic lobule-alveolar growth factor, which employs the first detectable signs of lobule development as a criterion for positive response, found that fresh anterior pituitaries from pregnant cattle gave positive results whereas lipid extracts be-

Received for publication December 14, 1942.

[1] Aided in part by a grant from the International Cancer Research Foundation. Contribution from the Department of Dairy Husbandry, Missouri Agricultural Experiment Station, Journal Series No. *877*.

lieved to contain mammogen I gave negative results. In subsequent work using a modified assay in which an injection of 75 I.U. of estrone was given to each animal in addition to the assay material, Mixner, Bergman and Turner (1942) obtained positive results with various protein extracts of cattle pituitary. It was concluded, therefore, that the mammogenic lobule-alveolar growth factor (mammogen II) was not associated with the lipid duct-growth factor.

Greep and Stavely (1941) reported their failure to obtain mammary growth in spayed hypophysectomized female rats with the waxy lipid fraction obtained by extracting female cattle pituitaries with a warm ether-alcohol mixture. However, they obtained mammary growth with both the extracted tissue residue and the unextracted desiccated pituitary. These positive results were presumably due to the same factor designated as mammogen II by Mixner and Turner.

Lewis, Gomez and Turner (1941), on the other hand, have reported that extracts of ether-fractionated lipid mammogen I caused growth of the mammary duct system of castrated male and hypophysectomized male and female rats. Gomez (1942) has reported that injections of the total amount of material obtained by extracting 20 to 50 mg. of fresh anterior pituitary (guinea pig) glands with a mixture of alcohol and ether resulted in growth of the mammary glands, equaling that of early pregnancy, in hypophysectomized-castrated guinea pigs of either sex.

More recently, we have been able to obtain only an occasional positive response with lipid extracts of anterior pituitary glands, assayed in the male mouse. A re-investigation of the extraction of cattle pituitary for the active factor involved in stimulation of mammary growth in the male mouse was accordingly undertaken.

EXPERIMENTAL PROCEDURE

It was decided that a number of available lipid extracts be re-assayed. Further, that new supplies of pituitaries be extracted and both lipid and protein fractions be assayed. One factor which has continually interfered with the assay of lipid fractions has been the persistent occurrence of a small percentage of positive responses in the control animals receiving the oil carriers of the hormone. The controls injected with olive oil in these experiments showed some positive responses. Of a total of 81 control mice, 6 showed positive responses or 7.4 per cent (table 1). Two other sources of error which might have influenced the previous results were considered. To prevent the use of syringes which might have been contaminated previously with estrogen or stilbestrol and not completely removed during their cleaning, new syringes were used exclusively and for one type of extract only. Secondly new rubber serum bottle stoppers were used exclusively to eliminate possible previous estrogen contamination.

The technique of assay in the normal male mouse (Lewis, Turner and Gomez, 1939) was used, the formation of end buds on any one or more of the mammary rudiments being taken as a criterion for positive response. The

TABLE 1. ASSAY OF LIPID EXTRACTS OF UNSELECTED CATTLE PITUITARIES

Extract	Carrier	Amount	No. of Mice	Positive Response	Negative Response
		mg.			
Control	Olive oil		81	6	75
No. 61	Olive oil	1 to 20	39	4	35
No. 66	Olive oil	0.05 to 20.0	60	1	59
No. 66	Propylene glycol	2.0 to 8.0	11	1	10

mice were injected subcutaneously on 6 successive days and killed on the seventh. Olive oil was used as a solvent for the lipid extracts with the exception of lipid extract No. 66, which was tried in both olive oil and propylene glycol (table 1). The fresh pituitary and proteinaceous extracts were suspended in water.

The cattle pituitaries were obtained from Swift and Co., Kansas City, Kansas. The anterior lobe only was used. Lot No. 14 was obtained from pregnant animals while lot No. 16 was from non-pregnant heifers. Lot No. 41 and lipid extracts No. 61, 66 and 67 were obtained from unselected cattle. The lipid extracts No. 14, 61, 66 and 67 were extracted by the same method, the latter two being obtained from the same shipment of pituitary tissue (Lot No. 41) but extracted at different times.

The wet, ground anterior pituitaries were dehydrated and defatted by mixing with 3 volumes of acetone. The acetone was immediately removed and the solids extracted 3 more times with acetone, followed by 2 washings with approximately 2 volumes of ether. The dehydrated pituitary material was exposed to the air in thin layers and stirred to facilitate drying, which was usually complete in about 5 to 10 minutes. The dry powder was designated 'acetone dried' and represented approximately 19 to 20 per cent of the wet weight of pituitary.

TABLE 2. ASSAY OF PITUITARY TISSUE FROM PREGNANT CATTLE, LOT NO. 14

Extract	Carrier	Amount	Fresh Tissue Equivalent	No. of Mice	Response Positive	Response Negative	% Positive
		mg.	*mg.*				
Fresh tissue	Water	125	125	13	11	2	85
		150	150	26	16	10	62
Lipid fraction No. 14	Olive oil	1	60	13	2	11	15
		2	120	13	1	12	8
		5	300	13	3	10	23
		12	720	15	1	14	7
		100	6000	11	1	10	9
Acetone-dried protein	Water	15	75	10	3	7	30
		30	150	13	8	5	61
		45	225	15	9	6	60
		60	300	13	9	4	70
Warm alcohol-ether re-extracted protein	Water	45[1]		14	8	6	57
		60		11	9	2	82
Warm alcohol-ether re-extracted lipid	Olive oil	10		8	1	7	12

[1] Castrated male mice used instead of normal males.

The acetone and ether supernatant fluids, containing the extracted water and lipid material, were pooled and filtered to remove suspended protein material. The fractionation was made in a manner similar to that previously reported by Bergman and Turner (1942). The filtrate was chilled to −10 to −15° C. overnight. This resulted in the settling out of a water-soluble residue. A preliminary assay of this residue from lot No. 41 showed a trace of physiological activity.

The supernatant fluid was vacuum-distilled (water pump) at a temperature of 30° C. to remove the acetone, ether and water. The material remaining in the distillation flask was extracted several times with ether. Roughly ⅕ to ¼ of the material was ether-insoluble. The ether-insoluble fraction consisted of a water-soluble reddish brown fluid and water-soluble solid material. The combined ether solution was again chilled to −10 to −15° C.

TABLE 3. ASSAY OF PITUITARY TISSUE FROM PREGNANT CATTLE, LOT NO. 14a

Extract	Carrier	Amount Injected	Fresh Tissue Equivalent	No. of Mice	Response Positive	Response Negative	% Positive
		mg.	*mg.*				
Fresh tissue	Water	40	40	6	3	3	50
		50	50	26	2	24	8
		60	60	10	6	4	60
		75	75	24	11	13	46
		100	100	22	10	12	45
		150	150	4	3	1	75
Lipid fraction No. 14a	Olive oil	5	300	10	2	8	20
		10	600	9	1	8	11
		20	1200	10	1	9	10
Protein fraction	Water	12	60	4	0	4	0
		24	120	4	0	4	0
		40	200	6	3	3	50
		48	240	4	0	4	0
		50	250	10	7	3	70
		60	300	10	10	0	100
		75	375	25	21	4	84

overnight and filtered in the cold. The filtrate was poured into a desiccator and the ether removed at low pressure. The final product was an amber colored, semi-waxy material having a pungent odor and representing from 1.5 to 1.7 per cent of the wet weight of the pituitary tissue (tables 1, 2 and 5).

The above extraction procedure differs from that used in the original experiments which produced an active crude lipid fraction (Lewis and Turner, 1938; 1939). An appreciable amount of water-soluble, ether-insoluble material which was originally included in the 'lipid' fraction is eliminated by this method of extraction. Lots No. 14(a) and 16 were accordingly extracted by the old ether-alcohol method. Two 15-minute extractions with ether-alcohol (1:3) mixture at about 55° C. were employed. The two ether-alcohol extractions were pooled and filtered, and the 'lipid' fraction obtained by vacuum distillation without further fractionation. By this method a larger yield of cruder material was obtained, representing 4.1 per cent of the wet pituitary weight. The yield of the protein fraction by this method was somewhat

TABLE 4. ASSAY OF PITUITARY TISSUE FROM NON-PREGNANT HEIFERS, LOT NO. 16

Extract	Carrier	Amount	Fresh Tissue Equivalent	No. of Mice	Response Positive	Response Negative	% Positive
		mg.	*mg.*				
Fresh tissue	Water	15	15	10	3	7	30
		20	20	8	3	5	38
		25	25	11	2	9	18
		30	30	9	4	5	44
		40	40	9	8	1	89
		50	50	21	9	12	43
		100	100	11	9	1	82
Lipid fraction No. 16	Olive oil	5	125	26	1	25	4
		10	250	15	0	15	0
		20	500	18	1	17	4
Alcohol-ether extracted protein	Water	5	28.5	10	6	4	60
		10	56.0	10	0	10	0
		15	85.5	18	4	14	22
		20	114.0	9	2	7	22
		30	171.0	17	9	8	53
		40	228.0	9	5	4	55
Soxhlet-re-extracted protein	Water	15		9	6	3	66

reduced. It represented 17.6 per cent of the fresh pituitary weight (table 3 and 4).

It was found in the course of the work that the dried protein fractions were retaining the bulk of the activity of the fresh pituitary, and since it was known that some lipid material remained unextracted in such fractions, especially those prepared by cold acetone and ether extraction, it became desirable to investigate the possibility that the activity of the protein fractions might be due to some unextracted lipid. Accordingly, portions of the dry protein fraction of lots No. 14, 16 and 41 were re-extracted with warm

TABLE 5. ASSAY OF UNSELECTED CATTLE PITUITARIES, LOT NO. 41

Extract	Carrier	Amount	Fresh Tissue Equivalent	No. of Mice	Response Positive	Response Negative	% Positive
		mg.	*mg.*				
Fresh tissue	Water	50	50	12	6	6	50
		100	100	14	8	6	57
Lipid fraction No. 67	Olive oil	5	300	14	2	12	14
		10	600	15	2	13	13
		20	1200	15	0	15	0
		100	6000	10	4	6	40
Acetone dried protein	Water	5	25	13	5	8	38
		15	75	13	7	6	54
		30	150	27	22	5	81
		60	300	13	11	2	85
Soxhlet re-extracted protein	Water	30		13	7	5	61
Soxhlet re-extracted lipid	Olive oil	10		5	0	5	0
		20		4	0	4	0

solvents. Lots No. 16 and 41 were Soxhlet-extracted for approximately 24 hours with ether-alcohol (1:3). Lot No. 14 was given two half-hour re-extractions with ether-alcohol at about 55° C. followed by two extractions with ether. The re-extracted protein and lipid fractions of each of these three lots were re-assayed, with the exception of the re-extracted lipid from lot No. 16. This lot, having originally been extracted with warm ether-alcohol yielded very little additional lipid, which was not assayed.

RESULTS

Re-assay of lipid extracts No. 61 and 66 and assay of the lipid fractions of lots No. 14, 14(a), 16 and 41 produced a few positive responses. However, there appeared to be no relation between these positive responses and the amount of extract injected. Of special importance is the fact that the proportion of positive responses obtained with lipid extracts was not very much greater than the proportion of positive responses obtained in the control groups injected with olive oil (table 1). The importance of having simultaneous control groups receiving the oil carrier only is thereby emphasized.

Moreover, the positive responses obtained with lipid fractions generally represented very minimal stimulation, whereas instances of extensive stimulation were frequent in the groups injected with the fresh pituitary and protein fraction. The best response encountered with a lipid fraction, both with regard to percentage and degree of response, was the 40 per cent response obtained with 100 mg. of lipid No. 67 (table 5). This group had 9 controls simultaneously injected with olive oil, all of which were negative. Some degree of activity must therefore be attributed to this lipid fraction, although on consideration of the dosage administered, and the large equivalents of fresh pituitary, the responses obtained with the lipid fractions might possibly be due to estrogenic activity of the pituitary tissue.

By comparison of the assays of the fresh pituitary and proteinaceous extracts it will be seen that in the present experiments the bulk of the activity of the fresh tissue was recovered in the protein fractions. Nor did re-extraction of the protein fractions with warm solvents remove their activity.

In the fresh pituitary or protein fractions it was found that the percentage response to increasing dosages was not always very uniformly graded, particularly as the dosage increased above that required to give a 50 per cent response. Further, it was difficult to attain a full 100 per cent response, at least in the dosages tried. This may be a reflection of the crude pituitary protein preparations used and the consequent relatively large amounts of material required. At any rate, the assay of such crude pituitary protein preparations is only roughly quantitative, and it is necessary to administer a wide range of dosages and to accept as the unit assay dose the smallest amount of material required to give a 50 per cent response.

Since androgens are capable of causing mammary gland stimula-

tion, and since the present work was done with normal male mice, it became necessary to determine whether the activity of the protein fractions might be due to their gonadotropic activity. Accordingly, 14 castrated male mice were substituted for normal males on the 45 mg. dosage level of re-extracted protein No. 14, as indicated in table 3. It will be seen that a positive response was still obtained in the castrated males.

The presence of the duct-stimulating potency in the protein rather than the lipid fraction brings up the question of the possible identity of this factor with the lobule-alveolar factor (mammogen II). For the few pituitary preparations thus far compared by the two assay methods, the amounts of material required for assay response by each method have been quite comparable. However, final settlement of this question must await further extraction, purification and assay of the protein extract.

DISCUSSION

The experiments reported in this paper indicate that the mammary duct-growth stimulating factor designated as mammogen I is present in the protein fraction of the anterior pituitary rather than in the fraction extracted with lipid solvents such as acetone, ether and alcohol which previous work in this laboratory had indicated. In the crude separation of lipid and protein in the earlier work, it is quite possible that some proteinaceous material was carried over into the 'lipid' fraction.

In the present work it was observed that in the first acetone-drying procedure appreciable amounts of water-soluble, ether-insoluble material, some of which showed some activity, was extracted along with the true lipid. This ether-insoluble material was separated out by chilling and ether fractionation. The inclusion of protein material in the 'lipid' fraction might explain results with the early extraction procedures, as well as positive results in hypophysectomized animals.

The possibility that known lipid-soluble hormones, particularly estrogen, may be present in the pituitary gland and may account for the previously reported effectiveness of the lipid extracts, has also been suggested. As mentioned above, some estrogenic activity had previously been reported (Lewis and Turner, 1939) in some active lipid extracts, although, it was felt, in insufficient amounts to account for the mammary growth-stimulating potency observed. Unlike the presence of protein, the presence of estrogen might explain the previous positive results with ether-fractionated lipid extracts. This is a possibility, especially where positive results were obtained with large amounts of the lipid fraction.

The occurrence of occasional positive responses in control mice injected with the olive oil carrier (table 1) has already been pointed out. The extent to which this factor influenced earlier results is, unfortunately, undeterminable.

SUMMARY

The extraction of cattle anterior pituitary for the active factor involved in the stimulation of mammary duct growth in the male mouse was re-investigated. Whereas previous work was interpreted as indicating that the factor is lipid in nature, in the present work, using improved extraction procedures, the bulk of the activity of the fresh pituitary was recovered in the protein fraction rather than in the lipid fraction. A discussion of various possible explanations is presented.

REFERENCES

BERGMAN, A. J., AND C. W. TURNER: *Mo. Agr. Exper. Sta. Res. Bul.* 356. 1943.
GOMEZ, E. T.: *Endocrinology* 31: 613. 1942.
GREEP, R O., AND H. E. STAVELY: *Endocrinology* 29: 18. 1941.
LEWIS, A. A., AND C. W. TURNER: *Proc. Soc. Exp. Biol. & Med.* 39: 435. 1938.
LEWIS, A. A., AND C. W. TURNER: *Mo. Agr. Exp. Sta. Res. Bul.* 310. 1939.
LEWIS, A. A., E. T. GOMEZ AND C. W. TURNER: *Endocrinology* 30: 37. 1937.
LEWIS, A. A., C. W. TURNER AND E. T. GOMEZ: *Endocrinology* 24: 157. 1939.
MIXNER, J. P., A. J. BERGMAN AND C. W. TURNER: *Endocrinology* 31: 461. 1942.
MIXNER, J. P., A. A. LEWIS AND C. W. TURNER: *Endocrinology* 27: 888. 1940.
WHITE, A.: *Annals of New York Acad. Sci.* 43: 341. 1943.

EVIDENCE OF HYPOTHALAMIC CONTROL OF HYPOPHYSEAL GONADOTROPIC FUNCTIONS IN THE FEMALE GUINEA PIG[1]

F. L. DEY

From the Institute of Neurology, Northwestern University Medical School
CHICAGO, ILLINOIS

It has been shown that the hypothalamus is connected with the posterior lobe of the hypophysis by means of a large unmyelinated fiber tract that runs by way of the infundibular stem. The components of this tract play an important rôle in regulating the secretory activity of the neural hypophysis (Fisher, Ingram and Ranson, 1938). A few fibers diverge from this tract or from the neural hypophysis and terminate within the anterior lobe of the pituitary (Croll, 1928; Brooks, 1938; Brooks and Gersh, 1937–38; and Rasmussen, 1938), but the number of fibers that do so is so small that it is doubtful whether they play a significant rôle in innervating the parenchyma of the anterior lobe. With the exception of these few fibers, no connection between the hypothalamus and the anterior lobe of the hypophysis has been demonstrated anatomically.

Electrical stimulation of the hypothalamus or of the hypophyseal stalk in female rabbits and cats quite uniformly results either in ovulation or pseudopregnancy (Marshall and Verney, 1936; Harris, 1936–37; Haterius, 1937; and Haterius and Derbyshire, Jr., 1937), while lesions of the hypothalamus may result in genital atrophy in the dog (Bailey and Bremer, 1921; Biggart and Alexander, 1939). The effect of transection of the hypophyseal stalk upon the gonadotropic activity of the pituitary, however, varies with the species of animal used and the investigator doing the work (Hinsey and Markee, 1933; Brooks, 1938; Brooks and Lambert, 1939; Richter, 1933; Mahoney and Sheehan, 1936; Keller and Hamilton, Jr., 1937; Dempsey, 1939; and Dempsey and Uotila, 1940). It is apparent that there is no uniformity of opinion concerning the influence that the hypothalamus may exert over the functions of the anterior lobe of the pituitary.

Investigations carried out in our laboratory have shown that lesions placed in the hypothalamus of female guinea pigs may cause genital disturbances in a small percentage of the cases (Dey, Fisher, Berry and Ranson, 1940; Dey, 1941). The lesions that cause these disturbances have a characteristic location and it was thought that a

Received for publication April 22, 1943.
[1] Aided by a grant from the Committee for Research in Problems of Sex, National Research Council.

description of them might prove helpful in future studies on this problem. In this paper the hypothalamic lesions causing genital disturbances are described and compared with other hypothalamic lesions that do not alter the sex cycle. In addition, the results of more recent experiments, in which lesions were made directly within the hypophysis, are presented.

METHOD

The brains of 83 female guinea pigs were utilized in this study. Seventy of the brains were from animals that had lesions in various parts of the hypothalamus, while the remainder were from animals that had lesions in the pituitary gland. The lesions were made with the aid of the Horsley-Clarke stereotaxic instrument according to the method previously described (Dey, Fisher, Berry and Ranson, 1940; Dey, Leininger and Ranson, 1942). Daily observations of the animals were made both before and after the operation and the condition of the vaginal membranes was recorded. The animals that had normal cycles following the operation were observed 150 days or more before they were killed. The animals with disturbances of the sexual cycle were usually autopsied after the disturbance was well established. For instance, the animals in which the vaginal membranes remained constantly closed were killed from 54 to 211 days after the last period of opening of the vaginal membrane.

The animals were killed by an overdosage of pentobarbital sodium and the brains were fixed in formalin. The hypothalami of thirty of the brains were embedded in paraffin and cut into serial sections 10 μ in thickness; every tenth section was stained by the Weil method and each next following section was stained with cresyl violet. The remainder of the hypothalami (including all the brains with lesions in the pituitary gland) were embedded in nitrocellulose and cut into sections 30 μ in thickness; every fourth section was stained by the Weil method and each next following section was stained with cresyl violet.

The ovaries were fixed in formol-Zenker fluid, embedded in paraffin and sectioned serially at 10 μ. The sections were stained with hematoxylin and eosin.

RESULTS

Animals with lesions in the hypothalamus. There were 70 animals with lesions in the hypothalamus. Nine of the animals ceased having estrous cycles after the operation, and the genitalia atrophied. The vaginal membranes of 19 animals remained constantly open and the uteri and external genitalia increased markedly in size. The 42 remaining animals showed cyclic genital changes which were regular in all except 6 animals. A more complete description of the genital changes that follow hypothalamic lesions has been presented (Dey, 1941).

The brains of 7 of the 9 animals in which the vaginal membranes remained constantly closed were suitable for the studies that we wished to make. The lesions of the 7 brains were all situated within the median eminence at the region of junction of the hypophyseal

stalk with the hypothalamus. In 4 of the brains the lesions completely surrounded this area of junction so that the hypophysis proper, most of the pars tuberalis and a small portion of the median eminence were completely severed from any connection with the hypothalamus. In the 3 remaining animals, the anterior portion of the lesions failed to reach the floor of the hypothalamus so that a slight connection remained between the ventral portion of the anterior hypothalamus and the rostro-ventral portion of the median eminence. Farther caudalward, however, the median eminence was more severely involved so that all dorsal, lateral and caudal connections to the stalk of the pituitary were severed. Direct damage to the anterior lobe of the pituitary gland occurred in only one of the 7 animals studied and in this animal the damage was very slight.

The lesions in the 19 animals in which the vaginal membranes remained constantly open were situated in the region of the optic chiasma and frequently extended caudally to the anterior end of the median eminence. In one animal the lesion included a portion of the anterior end of the median eminence but the areas dorsal, lateral and caudal to the junction between the hypothalamus and the infundibular stalk were intact. The lesions were large and destroyed a considerable portion of the anterior hypothalamic and supraoptic nuclei. We have not been successful in localizing more closely the area of destruction which is responsible for the altered gonadotropic functions.

There were 42 animals that continued to have regular estrous cycles following the operation. The lesions in these animals varied in location but in only 13 of the animals were the connections between the hypothalamus, the median eminence and the infundibular stalk damaged. This damage was slight except in 4 animals. In one of the 4 exceptions the lesion was unilateral and destroyed all of the lateral connections to the infundibulum on that side. In a second animal there were 2 lateral lesions, one on either side of the median eminence, that destroyed a considerable portion of the lateral connections between the hypothalamus and the median eminence but caused no direct damage to the median eminence itself. In the 2 remaining animals the lesions caused considerable damage to the median eminence so that the hypophyseal stalks were almost completely severed from the hypothalamus. In one animal, only a small portion of the caudal end of the median eminence was spared, while in the second, slight anterior and caudal remnants remained uninjured. The former animal had regular cycles and the ovaries were normal in appearance. The latter animal had cycles that were irregular in length, and usually greatly prolonged. The animal was killed after the vaginal membrane had been closed for a period of 24 days and the ovaries were quite atrophic and showed little evidence of follicular development.

Two of the animals that had regular cycles had lesions in the mammillary bodies. Both animals bred and bore young and the ovaries

were found to be normal at autopsy. The lesions were situated at the ventral surface of the mammillary complex and extended to the dorsal surface of the underlying hypophyses. In each case, the damage to the hypophysis was not extensive but the neural stalk was completely interrupted immediately rostral to the point at which it widens into the neural process. The extensive atrophy in the neural processes and the paucity of cells in the supraoptic nuclei verify the completeness of the transections. In these 2 animals, therefore, the connections of the hypothalamus with the median eminence and the rostral end of the stalk were left intact but the innervation to the neural process was destroyed.

Animals with lesions in the hypophysis. There were 14 animals in which lesions were placed directly within the hypophysis. The lesions varied in size—from ⅓ to twice the size of those placed in the hypothalamus of the animals of the preceding group. (See Dey, Leininger and Ranson, 1942, for the technique employed in making these lesions.) Nine of the animals had regular ovarian cycles following the operation, 3 had cycles that were irregular and in 2 animals the vaginal membranes remained constantly closed and the genitalia atrophied. The ovaries of the 2 latter animals were atrophic and contained but a very few follicles.

The lesions in 13 of the animals have been studied. The extent of the damage to the parenchyma of the anterior lobe varied from a slight amount to a destruction of ⅔ or more of the entire gland. The lesions frequently spread to the overlying mammillary complex. Contrary to what one might expect, the occurrence of the genital disturbances could not be correlated with a more extensive damage to the anterior lobe of the pituitary. In fact, the animal with the most extensive damage to the anterior lobe had regular ovarian cycles and became pregnant after the operation. It should be emphasized, however, that in this animal the anterior end of the hypophysis appeared to be quite normal and the neural stalk was not damaged until after it had passed through this area of normal glandular tissue. Two levels are shown in figure 1, A and B.

The lesions in the 2 acyclic animals extended into the hypothalamus and destroyed a considerable amount of the tuberal portion of the hypothalamus. In one animal, only a very slight connection remained between the ventral portion of the median eminence and the anterior hypothalamus. All dorsal, lateral and caudal connections between the hypothalamus and the median eminence were destroyed. Farther caudalward, the neural stalk was completely interrupted and the anterior end of the hypophysis was damaged, as is seen in figure 2, A. It should be noted that the major portion of the anterior lobe of this animal was not damaged by the lesion (fig. 2, B). The lesion in the second acyclic animal completely destroyed the median eminence and a large portion of the hypophysis.

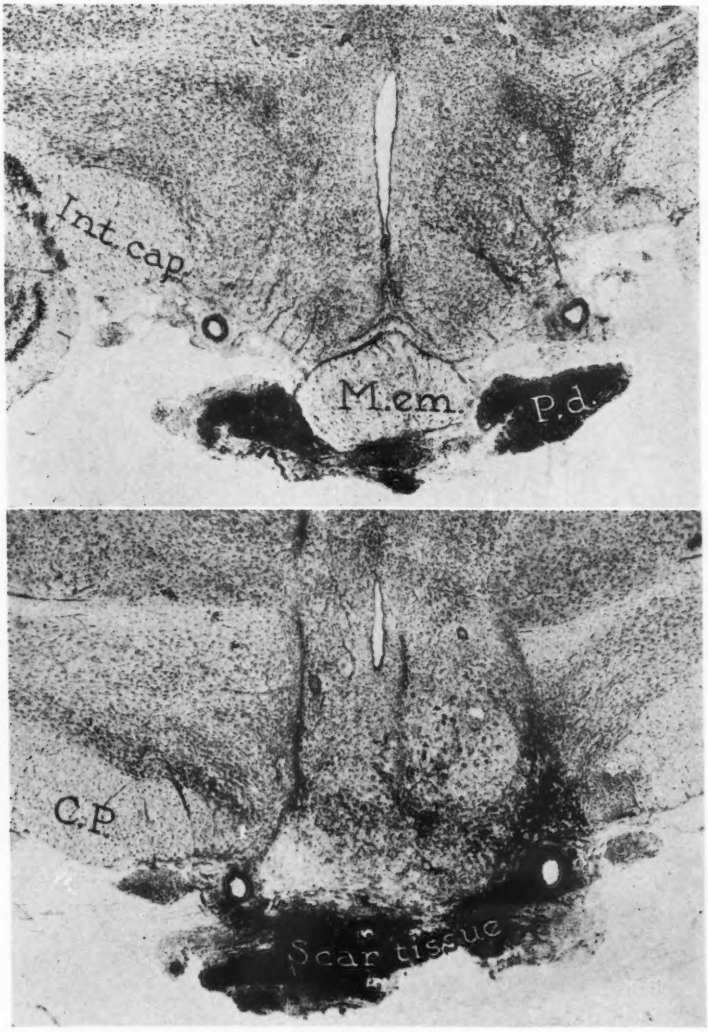

Fig. 1. Sections through the brain and hypophysis of a guinea pig that had normal estrous cycles postoperatively. Section A (*upper*) was made at the region of junction of the median eminence with the infundibular stem. Section B (*lower*) was from a level approximately half-way through the hypophysis on the rostral-caudal axis. The median eminence and anterior end of the hypophysis were uninjured in this animal, but approximately the caudal two-thirds of the pars distalis was destroyed. C. P., cerebral peduncle; Int. Cap., internal capsule; M. em., median eminence; P. d., pars distalis.

DISCUSSION

The observations made in this study indicate that the anterior hypothalamus and the median eminence may be concerned in the

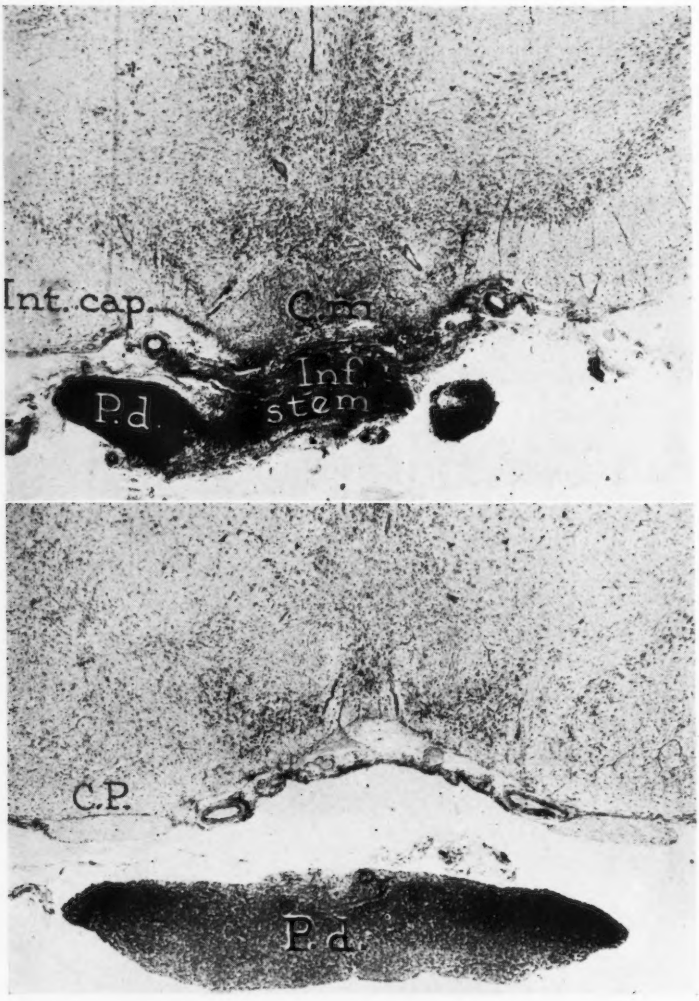

Fig. 2. Sections through the brain and hypophysis of a guinea pig that ceased having estrous cycles postoperatively. Section A (*upper*) is through the infundibular stem at the caudal end of the lesion. Section B (*lower*) was at a level midway through the hypophysis. The median eminence was almost completely destroyed in this animal, but only slight damage occurred to the anterior end of the pars distalis. C. m., mammillary bodies; C. P., cerebral peduncle; Inf. stem, infundibular stem; Int. cap., internal capsule; P. d., pars distalis.

regulation of pituitary gonadotropic functions in the female guinea pig. Destruction of these areas by electrolytic lesions interfere with normal sex cycles.

Lesions placed in the anterior hypothalamus at the caudal end of

the optic chiasma apparently interfere with the liberation of luteinizing hormone. The hormonal imbalance thus created results in marked follicular development in the ovaries and extensive hypertrophy of the external genitalia. We have been unable to localize more closely the area of destruction responsible for this disturbance, although in more recent experiments we have been able to reproduce this disturbance almost at will by placing large, bilateral lesions at the caudal end of the optic chiasma.

Lesions that destroy the median eminence result in genital atrophy. Our findings in this respect are by no means original. As early as 1920, Camus and Roussy reported that "total or almost total" removal of the hypophysis of the dog caused no disturbance of genital function but that extensive cauterization of the parahypophyseal regions caused genital atrophy. Other investigators have reported similar findings but it is difficult to determine from their publications the exact portion of the hypothalamus that was involved in the lesion.

The gonadotropic disturbances that occurred in our series cannot be attributed to direct damage to the anterior lobe of the hypophysis since in only one of the animals could such damage be detected. Neither can the dysfunction be attributed to electrolytic changes within the pituitary since direct hypophyseal lesions that destroyed up to two-thirds of this structure failed to abolish sexual cycles except when the median eminence and associated hypothalamic areas were also involved in the lesion.

At present we can offer no explanation for the hypophyseal dysfunction that occurred in the animals of our series. Previous investigators attempted to explain the hypophyseal dysfunction resulting from hypothalamic or parahypophyseal injury as being due either to an interruption of nerve fibers that pass to the hypophysis by way of the median eminence and stalk or to an interruption of the blood supply that reaches the hypophysis by way of the hypophyseal stalk. There is some doubt as to whether either of these explanations is correct. Dempsey (1939) and Leininger and Ranson (in press) have found that normal sex cycles may occur in the female guinea pig after transection of the hypophyseal stalk. Moreover, we have found that lesions that destroy the stalk, at or immediately after its junction with the hypophysis, do not alter the sex cycle. These experiments suffice to show that interruption of the vascular and nervous components is not incompatible with normal sex cycles in the female guinea pig. The explanation for the hypophyseal dysfunction that follows hypothalamic or parahypophyseal injury must await further experimentation.

SUMMARY

Marked gonadal changes occurred in 28 of 70 mature female guinea pigs after lesions were placed in the hypothalamus.

Nineteen animals were apparently unable to secrete luteinizing

hormone postoperatively. The hormonal imbalance thus created resulted in marked ovarian follicular development and genital hypertrophy. The lesions in this group of animals were large, bilateral and situated at the caudal end of the optic chiasma.

Nine animals were acyclic postoperatively and showed marked genital atrophy. The lesions were found to have destroyed a major portion of the median eminence. Direct damage to the anterior lobe of the hypophysis occurred in only one animal.

The lesions of the 42 remaining animals that showed normal estrous cycles postoperatively, destroyed various portions of the hypothalamus but in only 2 animals was there extensive damage to the median eminence and never was this structure completely destroyed.

Lesions placed directly within the hypophysis abolished sexual cycles in only 2 of 14 operated animals even though as much as two-thirds of the hypophysis was destroyed. The lesions of the 2 exceptional animals destroyed the median eminence and neighboring hypothalamic structures in addition to injuring the anterior lobe.

The results of these experiments indicate that the hypothalamus and median eminence may be concerned in the regulation of pituitary gonadotropic functions. It seems unlikely that they exert this control by nerve fibers that pass by way of the hypophyseal stalk.

REFERENCES

BAILEY, P., AND F. BREMER: *Arch. Int. Med.* 28: 773. 1921.
BIGGART, J. H., AND G. L. ALEXANDER: *J. Path. & Bact.* 48: 405. 1939.
BROOKS, C. M.: *Am. J. Physiol.* 121: 157. 1938.
BROOKS, C. M., AND I. GERSH: *Anat. Rec.* 70: 10, supplement 3. 1937–38.
BROOKS, C. M., AND E. F. LAMBERT: *Am. J. Physiol.* 128: 57. 1939.
CAMUS, J., AND G. ROUSSY: *Endocrinology* 4: 507. 1920.
CROLL, M. M.: *J. Physiol.* 66: 316. 1928.
DEMPSEY, E. W.: *Am. J. Physiol.* 126: 758. 1939.
DEMPSEY, E. W., AND U. U. UOTILA: *Endocrinology* 27: 573. 1940.
DEY, F. L.: *Am. J. Anat.* 69: 61. 1941.
DEY, F. L., C. FISHER, C. M. BERRY AND S. W. RANSON: *Am. J. Physiol.* 129: 39. 1940.
DEY, F. L., C. R. LEININGER AND S. W. RANSON: *Endocrinology* 30: 323. 1942.
FISHER, C., W. R. INGRAM AND S. W. RANSON: Diabetes Insipidus and the Neurohormonal Control of Water Balance. Edwards Brothers, Inc., Ann Arbor, 1938.
HARRIS, G. W.: *J. Physiol.* 88: 361. 1936–37.
HATERIUS, H. O.: *Cold Spring Harbor Symposia on Quantitative Biology* 5: 280. 1937.
HATERIUS, H. O., AND A. J. DERBYSHIRE, JR.: *Am. J. Physiol.* 119: 329. 1937.
HINSEY, J. C., AND J. E. MARKEE: *Am. J. Physiol.* 106: 48. 1933.
KELLER, A. D., AND J. W. HAMILTON, JR.: *Am. J. Physiol.* 119: 349. 1937.
LEININGER, C. R., AND S. W. RANSON: In press. 1943.
MAHONEY, W., AND D. SHEEHAN: *Brain* 59: 61. 1936.
MARSHALL, F. H. A., AND E. B. VERNEY: *J. Physiol.* 86: 327. 1936.
RASMUSSEN, A. T.: *Endocrinology* 23: 263. 1938.
RICHTER, C. P.: *Am. J. Physiol.* 106: 80. 1933.

EFFECTS OF THYROTROPIC HORMONE, GONADOTROPIC FACTOR, PITUITARY GROWTH SUBSTANCE AND INSULIN UPON THE PHOSPHATASE CONTENT OF RAT FEMURS[1]

C. H. WHICHER AND E. M. WATSON
From the Department of Pathological Chemistry
University of Western Ontario
LONDON, CANADA

IN previous reports it was shown that certain hormonal preparations when injected into albino rats apparently produced changes in the phosphatase content of the femurs (Williams and Watson, 1941). For example, repeated small doses of parathyroid extract caused a temporary increase of the phosphatase of the diaphyses without demonstrable alteration of this enzyme in the epiphyses. Large single doses of the extract caused an increase, followed by a decrease, of the phosphatase of the diaphyses while only a decrease in the epiphyses was noted. The injection of adrenal cortical extract was followed by a reduction of the phosphatase of both the diaphyses and the epiphyses, the effect being more marked in the latter. Desoxycorticosterone acetate exhibited a tendency to raise the phosphatase activity of the bones. The administration of progesterone, testosterone, estradiol, thymus extract and thyroxin was followed by increases of the phosphatase content of the femurs.

The additional observations appearing below represent a continuation of these studies with special reference to the effects of hormones derived from the anterior pituitary gland.

METHODS

The methods employed were essentially the same as those described in connection with the preceding series of experiments (Williams and Watson, 1941). One hundred and forty albino rats ranging in age from 50 to 100 days were used in the present studies. Litter mates of the same sex and as nearly the same weight as possible were chosen. Some of the animals of each group received by injection the preparation under investigation; the others served as controls. At definite intervals after the injections, the animals were killed by means of chloroform and the femurs dissected out. The diaphyses and the epiphyses were divided, separately, into small particles and autolysed for two days with water in order to extract the phosphatase. Then the frag-

Received for publication May 11, 1943.
[1] This work was supported by a grant from the Associate Committee on Medical Research of the National Research Council, Ottawa, Canada.

ments of bone were filtered off and the enzyme extract dialysed for 36 hours in a collodion sac against three changes of distilled water. The extract was filtered and subsequently diluted to 25 cc. Thereupon it was ready for analysis. The remaining bony fragments were treated with ether to remove the fat, then dried and weighed.

The estimation of the relative concentration of the alkaline phosphatase in the extracts of the bones was accomplished by a modification of the method described by Bodansky (1937), under optimal conditions of pH, α-amino acid and magnesium ion concentrations. The final result was expressed as a percentage of comparable values obtained from the control animals on the basis of the reaction velocity per gm. of dry, fat-free bone.

DATA

Thyrotropic factor of the anterior pituitary. The subcutaneous injection of 1 cc. of an anterior pituitary extract[2] containing the thyrotropic factor caused an initial decrease of the bone phosphatase followed by a considerable increase. The maximal increment involving both the diaphyses and the epiphyses occurred 48 to 60 hours after the injection, as exemplified in table 1. This action of the thyrotropic hormone upon the phosphatase of the femurs is similar to that found by Williams and Watson (1941) for thyroxin.

TABLE 1. EFFECT OF THYROTROPIC HORMONE AND IODIDES UPON THE PHOSPHATASE CONTENT OF THE FEMURS OF RATS

Hours after Injection	Relative Phosphatase Activity, %					
	Thyrotropic hormone		Potassium iodide		Sodium iodide	
	Diaphyses	Epiphyses	Diaphyses	Epiphyses	Diaphyses	Epiphyses
12	97	98	140	105	98	129
24	73	77	140	118	90	95
36	109	100	121	186	80	81
48	183	114	82	116		
60	102	172	114	109		
72	102	70				

Silberberg and Silberberg (1938, 1939) reported that solutions of potassium iodide, when injected intraperitoneally into immature guinea pigs, produced stimulation of the growth of cartilage comparable to that observed after the administration of thyroid substance. The proliferation of the euhyaline cartilage due to potassium iodide did not occur apparently through the mediation of the thyroid gland since it was found in thyroidectomized animals.

The intraperitoneal injection of aqueous solutions containing 100 mg. of potassium iodide caused an increase of the bone phosphatase which was apparent after 12 to 24 hours. This increase was maintained in both the diaphyses and epiphyses as long as 48 hours. The administration of sodium iodide in a dose of 90 mg. was followed by an

[2] Supplied by Ayerst, McKenna and Harrison, Ltd., Montreal, Canada.

increase of the epiphyseal phosphatase only but not of the same degree as was noted after the use of potassium iodide (table 1). Neither potassium nor sodium chloride produced any significant variation of the bone phosphatase.

Gonadotropic factor of the anterior pituitary. The dose of a preparation containing the gonadotropic factor[3] administered subcutaneously was 1 cc. (800 Collip units). The phosphatase content of both the diaphyses and the epiphyses was increased thereby but the increase appeared sooner and remained longer in the case of the diaphyses than in that of the epiphyses (table 2). These findings bear a certain resemblance to those previously obtained with testosterone and estradiol (Williams and Watson, 1941).

TABLE 2. EFFECT OF GONADOTROPIC FACTOR AND PITUITARY GROWTH SUBSTANCE UPON THE PHOSPHATASE CONTENT OF THE FEMURS OF RATS

Hours after Injection	Relative Phosphatase Activity, %					
	Gonadotropic factor		Growth substance I		Growth substance II	
	Diaphyses	Epiphyses	Diaphyses	Epiphyses	Diaphyses	Epiphyses
12	155	68	110	212	104	94
24	119	114	83	102	113	167
36	145	207	105	200	112	154
48	158	64	105	107	108	97
60	134	105				

Pituitary growth substance. The results obtained with pituitary extracts purported to contain the growth-stimulating principle were inconsistent. For example, one product[4] in doses of 0.75 to 2.0 cc. seemed to produce a pronounced increase of the bone phosphatase in some experiments and a decrease in others.

Another preparation[5] which was claimed to possess in addition to the growth hormone unknown amounts of the thyrotropic and gonadotropic factors gave much more uniform results although discrepancies were encountered with this product also, especially with regard to the diaphyses. So it can be stated that the preparations of growth hormone employed in these experiments failed to produce uniform effects upon the phosphatase content of the bones. The figures shown in table 2 represent the averages of all the results obtained with the two preparations of pituitary growth substance used in the experiments.

Insulin. In view of the rôle of insulin in carbohydrate metabolism wherein phosphatase functions evidently as one of the important enzymes, it was decided to determine, if possible, if insulin would ex-

[3] Supplied by Ayerst, McKenna and Harrison, Ltd., Montreal, Canada.

[4] Phyone, provided by Wilson and Company, Chicago, Ill.

[5] Anterior Pituitary Extract, furnished by E. R. Squibb and Sons, New York City, N. Y.

ert any effect upon the phosphatase content of the bones. The theory relating to the enzymatic control of ossification recently propounded by Gutman and associates (1942) attached special interest to this aspect of the problem. Forty units of protamine zinc insulin were injected and the animals killed after 12, 18 and 24 hours. The failure of this amount of insulin to cause any definite or regular change in the bone phosphatase may be related, perhaps, to the known resistance of the albino rat to the hypoglycaemic action of insulin.

SUMMARY

The effects of various extracts of the anterior pituitary gland, insulin and iodides upon the phosphatase content of the femurs of rats have been observed.

Both the thyrotropic hormone and the gonadotropic factor, injected subcutaneously, caused an increase of the phosphatase content of the diaphyses and the epiphyses.

The preparations of the growth-promoting factor of the anterior pituitary which were used did not exert a consistent influence upon the bone phosphatase. In some experiments they produced an increase in others a decrease and in still others no change whatsoever.

Insulin did not cause any modification in the phosphatase content of the bones of the rats.

Potassium iodide produced an increase of the phosphatase of the femurs.

The authors gratefully acknowledge the valuable assistance in this work supplied by Dr. H. L. Williams.

REFERENCES

BODANSKY, O.: *J. Biol. Chem.* 118: 341. 1937.
GUTMAN, A. B., F. B. WARRICK AND E. B. GUTMAN: *Nature* 95: 461. 1942.
SILBERBERG, M., AND R. SILBERBERG: *Growth* 2: 369. 1938.
SILBERBERG, M., AND R. SILBERBERG: *Arch. Path.* 28: 846. 1939.
WILLIAMS, H. L., AND E. M. WATSON: *Endocrinology* 29: 250. 1941.
WILLIAMS, H. L., AND E. M. WATSON: *Endocrinology* 29: 258. 1941.

GROWTH AND METABOLISM OF YOUNG HYPOPHYSECTOMIZED RATS FED BY STOMACH TUBE[1,2]

LEO T. SAMUELS, ROGER M. REINECKE AND KENNETH L. BAUMAN

From the Division of Physiological Chemistry, Department of Physiology, University of Minnesota

MINNEAPOLIS, MINNESOTA

It has not been determined whether the young hypophysectomized rat has the capacity to utilize more food than it would voluntarily consume and consequently to grow. Cessation of growth after hypophysectomy was first experimentally demonstrated by Aschner (1912) and later confirmed by the studies of Smith (1930). Lee and Ayres (1936) demonstrated that hypophysectomized rats allowed to eat *ad libitum* lost more weight, more nitrogen and less fat than controls maintained on the same quantity of food. Lee and Schaffer (1934) also found that the growth produced by pituitary extract was associated with increased storage of nitrogen and decreased storage of fat even though food intake was maintained at the level of that of the normal untreated controls. In each case, however, the level of food supplied was that of the animal with the lesser intake; these studies did not determine how the hypophysectomized rat would metabolize a food intake approaching normal.

In the present experiments food was provided in quantities which maintained steady, although subnormal, growth in controls. It was noted that the hypophysectomized rats continued to gain weight. They also stored small quantities of nitrogen and large amounts of fat. Apparently such animals could metabolize greater amounts of food than they would voluntarily eat, but the balance between different metabolic processes was changed.

METHODS

Rats from the colony of the Institute of Anatomy of this University were used in these studies. Only animals from litters which contained three or more rats of the same sex and similar body weight were utilized for the experiment. All rats were subjected to anesthesia and trephining of the sphenoid.

Received for publication May 19, 1943.

[1] This investigation was supported by a grant from the Committee on Research in Endocrinology, National Research Council.

[2] Assistance in the preparation of these materials was furnished by the personnel of the Works Project Administration, Official Project No. 165-1-71-124, Sub-project #355.

The pituitary gland was then removed by suction in at least one of each litter. The pituitary of one of the animals from each litter was not aspirated; instead, the incision was closed after simply exposing the gland; this was the 'fed control.' One of the other rats was then killed as the 'initial control' from which all changes due to hypophysectomy and to food intake could be determined. It was dissected according to the method of Jackson (1913).

The incisors of the remaining rats were then clipped with small bone forceps, and the animals were fed throughout the experiment solely by a stomach tube prepared from a No. 8 French rubber catheter. The teeth were clipped twice a week during the experiment.

The diet was prepared according to the methods described in a previous paper (Reinecke and Samuels, 1942). The animals were fed three times a day, at approximately 5-hour intervals. The amount per feeding was based upon the tolerance of the hypophysectomized animals, three feedings of 4 cc. each being enough to maintain a rate of weight gain of 2 to 3 gm. per day in a rat weighing 90 to 100 gm. When the control rats ceased to gain on a given intake of food, the amount per feeding was increased.

If the experimental period was considered sufficient or a hypophysectomized rat died, the littermates were killed, dissected and weighed in the same way as the initial controls. Then all the tissues from each animal, with the exception of the liver and the region of the sella turcica, were digested with sulphuric acid and analyzed according to methods previously described (Samuels, *et al;* 1942). The liver was analyzed separately by the same procedures.

The region of the sella turcica was removed from all animals and fixed in formalin. It was later sectioned serially, every tenth section mounted, stained with azo-carmine and examined for subdiaphragmatic pituitary gland. If any of this tissue was present the animal and its controls were discarded.

Twelve litters of rats from 4 to 6 weeks of age were started on the experiment. Animals from only five litters completed the experiment satisfactorily, however, the other groups being eliminated either because the hypophysectomized rats died too soon for the results to be significant, or because they were not completely hypophysectomized.

RESULTS

Growth of skeleton and musculature appeared to continue slowly in the hypophysectomized rats under the conditions of this experiment. In figure 1 the weights of various organs and certain chemical constituents, expressed as the percentage of the weight of the organ or constituent in the initial control, are plotted for the different pairs of fed animals against time as the ordinate. Small gains in weight were shown by a number of organs of the hypophysectomized rats. It will be observed that there was a relatively uniform increase in the weight of the femurs as the animals were fed for longer periods before dissection. The rate of gain was about 10 per cent of that of the controls. The uniformity of increase throughout the feeding period indicates that it was probably not due to residual hormone during the first few days. Similarly there was apparently a continued storage of nitrogen

at a low rate. This was more rapid, however, than the skeletal growth, representing approximately one-third of the increase found in the controls.

The organs which showed weight losses in spite of the heavy feeding were the adrenals, gonadal system, thyroids, spleen, and thymus, all of which have been shown to decrease in size in hypophysectomized rats on diets fed *ad libitum*.

The only tissues which showed a large increase in weight in the hypophysectomized rats were the fat depots (fig. 1). Apparently the

TABLE 1. CHANGES IN WEIGHT AND RATIO OF LIVER NITROGEN TO CARCASS N IN YOUNG FORCE-FED RATS

	Days on Exper.	Body Wt. Before Feeding	Body Wt. at Autopsy	Final Wt. Less G.-I. Contents	Ratio Liver N/Carcass N
		gm.	*gm.*	*gm.*	
1. Initial control	0	110.5	110.5	97.6	.044
2. Hypo.	15	104	111.5	100.3	.063
3. Initial control	0	107	107.0	101.1	.058
4. Hypo.	22	104	133.5	105.4	.062
5. Fed control	22	102	157.5	139.3	.060
6. Initial control	0	109	109.0	102.7	.043
7. Hypo.	32	97	120.0	102.5	.062
8. Fed control	32	93	136.5	122.3	.045
9. Initial control	0	102	102.0	94.3	.044
10. Hypo.	54	102	151.5	141.0	.077
11. Fed control	54	98	162.0	153.7	.041
12. Initial control	0	61.8	61.8	46.1	.060
13. Hypo.[1]	24	63	105.5	97.5	.070
15. Hypo.[1]	24	64	113.7	95.8	.106
14. Fed control	24	55	113.4	110.8	.056
16. Fed control	24	63	114.8	109.5	.049

[1] These rats had small fragments of anterior lobe tissue remaining in the region of the sella turcica. The series is included in the table because it illustrates that, while growth was greater in these animals, the relative distribution of nitrogen between liver and the remaining carcass and the nitrogen balance (see fig. 2) were similar to those of hypophysectomized rats. Data on organs of this series are not included in figure 1.

hypophysectomized animals used only small amounts of protein anabolically in the tissues in general but were quite able to deaminate amino acids and accumulate body fat.

The amount of liver fat was less, however, in the hypophysectomized rats than in the paired controls and therefore paralleled the rate at which fat must have been burned in the 2 groups. On the other hand, the ratio of liver nitrogen to carcass nitrogen was higher in the deficient animals than in the controls. This difference in the effect of pituitary removal on the liver and on the rest of the body was previously pointed out in balance studies on adult animals (Samuels, *et al.*, 1942).

In table 1 the changes in body weight of the different litters of rats

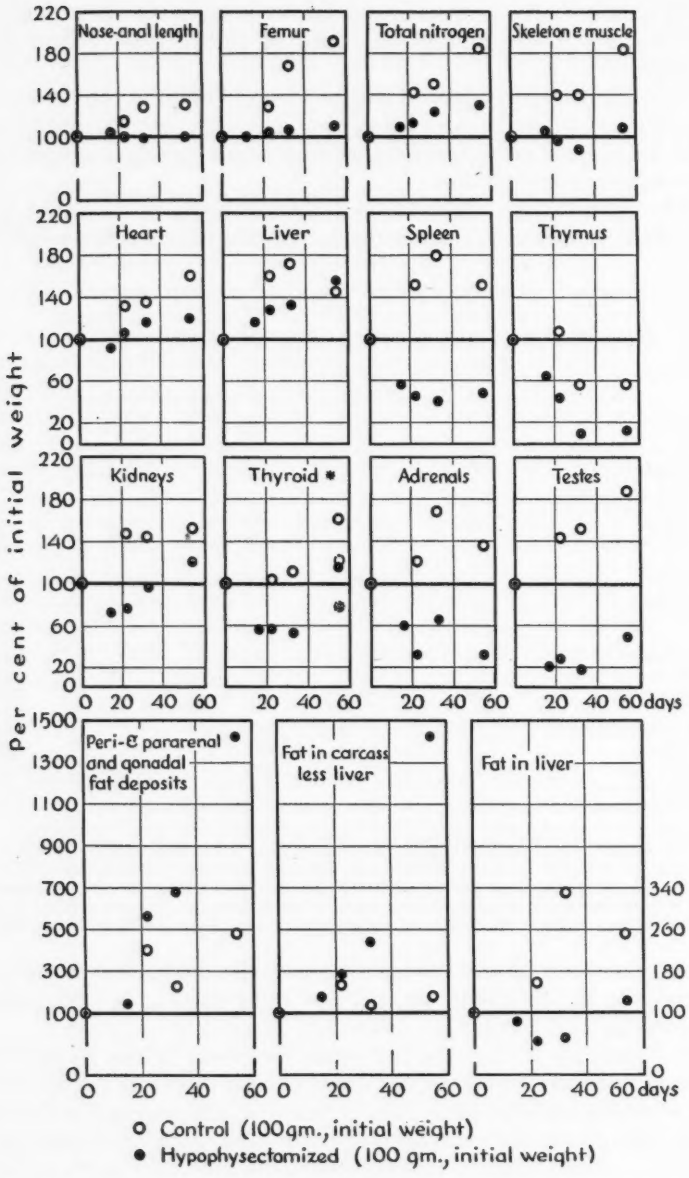

Fig. 1. Weight of certain organs and constituents expressed as a percentage of the weight found in the littermate killed at the beginning of the experiment. The values re palotted against the time the animals were on the experiment. In this way possible

are given. It will be noticed that the hypophysectomized rats did not gain as much weight as the control animals, even though they received the same amount of diet. A considerable part of this is accounted for by the differences in body composition.

The caloric balance for the three pairs of rats on which adequate data were obtained is given in table 2. The composition of the food administered, of the excreta, and of the carcasses before and after feeding have been expressed on a caloric basis, 4.1 calories being assumed per gm. of carbohydrate, 9.3 calories per gm. of fat and 25.6 calories per gm. of nitrogen. The hypophysectomized rats showed the lower total metabolic rate which would be anticipated.

TABLE 2. CALORIC BALANCE ON YOUNG FORCE-FED RATS

Rat Number	4	5	7	8	10	11
Type of Preparation	Hypophysectomized	Control	Hypophysectomized	Control	Hypophysectomized	Control
Days on diet	22	22	32	32	54	54
Total intake, calories	725.4	745.1	1053.9	1053.9	1822.5	1822.5
Unabsorbed food, calories						
Carbohydrate	27.5	19.3	37.8	27.3	74.0	47.2
Protein	21.5	14.6	24.3	17.5	41.1	25.9
Fat	72.1	50.9	65.2	47.4	138.9	86.3
Total unabsorbed, calories	121.1	84.8	127.3	92.2	254.0	159.4
Increase in N stored, calories	6.9	34.2	16.2	33.4	17.9	61.4
Increase in fat stored, calories	96.7	80.6	245.2	34.1	398.9	81.6
Total caloric increase	103.6	114.8	261.4	67.5	416.8	143.0
Calories used	500.7	545.5	665.2	802.0	1155.7	1520.1
Total metabolic rate Cal/day	22.8	24.8	20.8	25.1	21.4	38.2
Total metabolic rate[1] Cal/sq.m./day	10.3	10.7	10.0	11.6	9.3	12.1

[1] This is not basal metabolic rate, but the average total calories used in a day.

The nitrogen balance for the animals is given in figure 2. In the hypophysectomized rats the amount of nitrogen excreted in the urine was greater than that in the controls. The fecal nitrogen was also higher, but in both groups of animals the amount in the feces was such a small fraction of the daily nitrogen intake (13% for the hypophysectomized rats and 8% for the controls) that differences in digestion and absorption were not sufficient to account for the differences between the two groups of animals.

trends may be observed. Each dot represents one rat and where two dots lie on the same vertical axis, they represent littermates.

*The thyroid of the control rat in the series longest on the diet was very small, probably because of errors in dissection. The dotted circles represent the relative size of the fed control and hypophysectomized rats when compared with the mean weight of the thyroids of other rats of the same age.

Except in two or three isolated instances the collections for each 5-day period showed that a certain amount of the food nitrogen was unaccounted for in the urine and feces. This was much higher in the

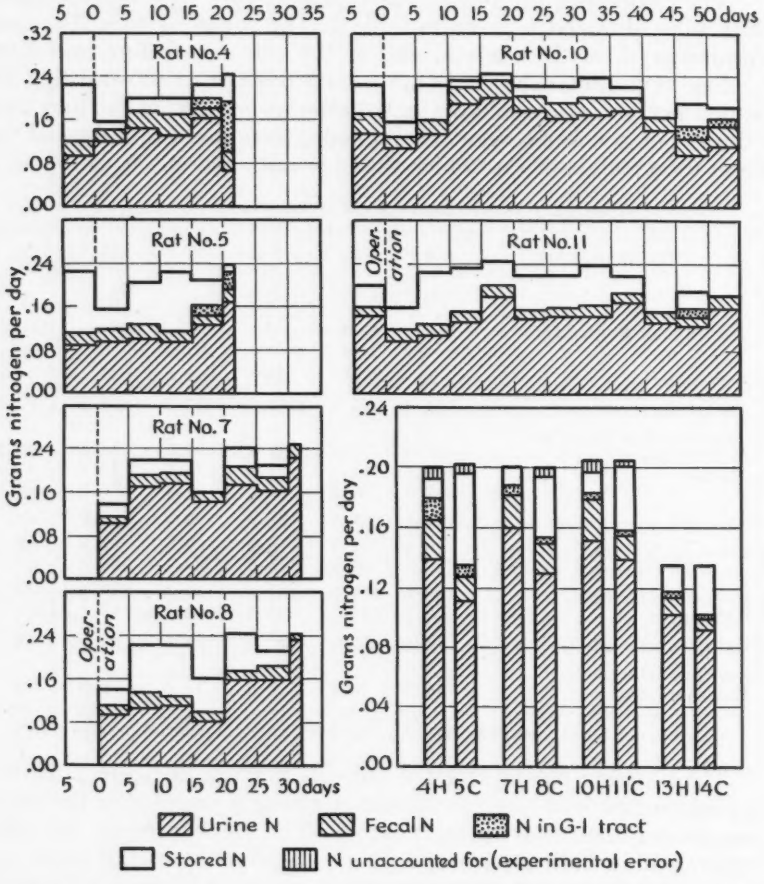
Rat No.4
Rat No.10
Rat No.5
Rat No.11
Rat No.7
Rat No.8
Oper-ation
Grams nitrogen per day
5 0 5 10 15 20 25 30 35
5 0 5 10 15 20 25 30 35 40 45 50 days
5 0 5 10 15 20 25 30 days
4H 5C 7H 8C 10H 11C 13H 14C
Urine N
Fecal N
N in G-I tract
Stored N
N unaccounted for (experimental error)

Fig. 2. Nitrogen balances of hypophysectomized and control rats during forced feeding. The numbers of the rats correspond to those given in table 1. Rats 4 and 5, 7 and 8, 10 and 11, 13 and 14 were paired litter mates. The graphs with days as abscissae represent the balances by 5-day periods. The bar graphs represent the distribution of the total nitrogen divided by the number of days the animal was on the experiment.

controls and coincided with the greater storage of nitrogen in these animals. Since the nitrogen unaccounted for in the urine and feces was fairly constant until a short time before the death of the deficient animals, this would appear to confirm the conclusions based on the graph

of the gains in carcass nitrogen, *i.e.*, that a slow storage of nitrogen continued in the hypophysectomized animals throughout the feeding period.

The fat absorption was quite high in all animals, being approximately 90 per cent complete. As in the case of fecal nitrogen, the fecal fat in the hypophysectomized rats was slightly higher. Apparently digestion and absorption were not limiting factors during most of the feeding period. The hypophysectomized animals, therefore, had a sufficient amount of food available for energy needs and for growth.

It would seem, therefore, that the removal of the pituitary gland does not seriously reduce the ability of young rats to assimilate food. It does reduce the proportion of dietary nitrogen used for protein synthesis, and increase that which is deaminated into forms primarily used for energy. Neither process, however, is wholly inhibited.

DISCUSSION

Apparently the hypophysectomized young rat does not suffer from any serious reduction in the absorptive capacity of the intestinal tract. The small reduction in absorption is probably associated with the general reduction in metabolic rate. The reduction in food intake which occurs when such animals are allowed to eat *ad libitum* must therefore be associated with changes in intermediary metabolism rather than in the intestinal tract.

The fact that there was only a slight difference in the absorption of the various foodstuffs while there was a great difference in body composition indicates that the paths of intermediary metabolism must be profoundly altered in the absence of the pituitary gland. Such a difference was indicated by the changes in composition of the paired-fed rats of Lee and Ayres (1936), but in this case it was not a question of the route by which excess foodstuffs were disposed of but the relative rate at which the endogenous compounds disappeared. Both these and the present experiments indicate the same thing: that less protein is used anabolically and more is deaminated in the absence of the pituitary secretions. This was also borne out by the changes in composition which Lee and Schaffer (1934) found in the carcasses of rats which had been injected with crude pituitary extracts but maintained on the same food intake as uninjected controls. Here there was a greater storage of protein and less storage of fat, the reverse of the picture found in the present experiment.

There are three possible explanations for this, any or all of which may play a part. The first possibility is that there is always a conversion of other food stuffs into fat, part of which is deposited in the storage tissues. In the absence of pituitary hormones the utilization of this stored fat is interfered with and protein must be used for energy instead. The second explanation is that the synthesis of protein is catalyzed by pituitary hormones. In its absence the amino acids are

not removed from the blood stream for this purpose with normal rapidity, and subsequent utilization for energy or fat formation is increased. The third condition under which such a balance might be brought about is a primary acceleration of deaminative processes and a consequent reduction in the amount of amino acids available for formation of structural protein.

The first two mechanisms seem the most likely to be involved. The changes in the liver would tend to indicate that fat metabolism might be depressed. The work with deuterium-containing fats has shown that there is a constant, although slow, replacement of fat in the depots, even when the net change is in the direction of reduced fat content. They also show that a considerable part of the depot fat is transported to the liver. The small amount of liver fat in contrast to the high amount of depot fat would indicate a reduction in this transport process.

The great reduction in growth of skeleton and musculature in the presence of available foodstuffs would favor the view that there is a reduction in the ability to synthesize structural elements. The fact that wounds heal readily in hypophysectomized animals indicates that this process is not completely inhibited however. The slow increase in carcass nitrogen and the gradual growth of some organs further supports the hypothesis that the removal of the pituitary gland eliminates a factor which catalyzes the anabolic process but is not essential for it.

The failure of the hypophysectomized rats to gain as much weight as the controls on the same food intake is more than accounted for by the differences in composition of the carcasses. One gram of fat is equivalent in calories to more than two grams of protein. The latter substance is also laid down with much larger amounts of water. Consequently, when a caloric balance is struck, as shown in table 2, it is found that the hypophysectomized rats with their high fat content, actually used less calories and stored more in body constituents than the heavier controls.

In conclusion, it would seem that the composition of the body as a whole indicates an increase in fat storage and a decrease in protein synthesis, while the composition of the liver bears a close relation to the mixture being oxidized, which is higher in protein and lower in fat than the controls.

SUMMARY

Young rats, 60 to 100 gm. in weight, were hypophysectomized and then maintained by stomach-tube-feeding on a food intake which caused growth in littermate controls. While such animals lost slightly more fat and nitrogen in the feces, the intestinal absorption was good in all cases.

The hypophysectomized rats showed a very slow increase in skeletal growth, 10 per cent of that in the controls, and a small increase in nitrogen storage, about 30 per cent of that in the controls.

These animals deaminated much more protein and stored more fat than the littermate controls. This accounted for their lower weight in spite of a lower metabolic rate.

The livers of the deficient rats did not vary in the same manner as the balance of the carcass, but instead reflected the mixture that was being burned. They contained less fat and as much or more protein than the livers of the controls.

REFERENCES

ASCHNER, B.: *Arch. f. d. ges. Physiol.* 146: 1. 1912.
JACKSON, C. M.: *Am. J. Anat.* 15: 1. 1913.
LEE, M. O., AND G. B. AYRES: *Endocrinology* 20: 489. 1936.
LEE, M. O., AND N. K. SCHAFFER: *J. Nutrition* 7: 337. 1934.
REINECKE, R. M., AND L. T. SAMUELS: *Endocrinology* 30: 687. 1942.
SAMUELS, L. T., R. M. REINECKE AND H. A. BALL: *Endocrinology* 31: 35. 1942.
SMITH, P. E.: *Am. J. Anat.* 45: 205. 1930.

EFFECT OF HYPOGLYCEMIA AND ANOXIA ON THE SURVIVAL PERIOD OF INFANT AND ADULT RATS AND CATS

HAROLD E. HIMWICH, JOSEPH F. FAZEKAS AND EDMUND HOMBURGER

From the Department of Physiology and Pharmacology, Albany Medical College, Union University

ALBANY, NEW YORK

THE literature contains a number of reports on the comparative effects of anoxia in infants and adults of the same species as well as between members of different species (Kabat, 1940; Fazekas, Alexander and Himwich, 1941; Himwich, Bernstein, Herrlich, Chesler and Fazekas, 1942). In general, it was found that newborn of all species examined possess greater resistance to anoxia than adults, a resistance dependent upon the greater capacity of the infant brain to withstand oxygen deprivation. Glucose (Himwich and Nahum, 1932), like oxygen, is an obligate of cerebral metabolism. We, therefore, extended this report beyond the study of anoxia to include the relative capacity of infants and adults in resisting hypoglycemia. In addition, the duration of survival of adult animals when exposed to the combined effects of anoxia with either hypoglycemia or hyperglycemia were observed. In previous observations of infant rats it was found that one-day-old rats survived 50 minutes in nitrogen but if insulin hypoglycemia was superimposed upon anoxia the survival period was shortened to 20 minutes. Eight-day-old rats survived 16 minutes in nitrogen but the intraperitoneal injection of glucose prolonged this period to 30 minutes. It will be seen that there are both similarities and differences in the reactions of the infant and adult rats.

METHOD

These experiments were divided into 4 parts. *a*) Infant and adult rats were subjected to hypoglycemia produced by various doses of insulin. Their survival time and blood sugar levels were determined. The animals were decapitated, and samples of blood collected in a pipette from the severed neck were analyzed for glucose by the method of Hagedorn and Jensen (1923). One cc. of blood was drawn from the adults and 0.2 cc. from the infants. *b*) The period of survival of adult cats injected with a toxic dose of insulin was studied. c) In order to evaluate the combined effects of either hypoglycemia or hyperglycemia with anoxia on the survival period of adult rats and adult and infant cats, the duration of survival of these animals in an atmosphere

Received for publication June 1, 1943.

of 4.5 to 5 per cent oxygen in nitrogen was ascertained. With this information the modifying action of insulin hypoglycemia and of hyperglycemia produced by the intravenous injection of glucose, 2 gm. per kg., was determined. *d*) Two groups of rats, one fed and the other fasted for 18 hours, were exposed to partial anoxia as in *c*. Samples of blood were collected and analyzed for glucose in order to determine the effect of fasting on blood-sugar level.

RESULTS

a) It was found that 37 newborn rats, weighing from 4 to 6 gm., survived a period of 10 to 16 hours even with large doses of insulin

TABLE 1. TOXICITY OF INSULIN IN ADULT CATS AND RATS

Units per Kg.	Number Injected	Number Succumbing Within 4 to 16 hr.	Number Succumbing Within 1 to 14 days
	Cats		
2.5	7	1	0
5.0	9	3	2
7.5	2	0	0
10.0	9	3	3
15.0	6	4	0
20.0	5	3	0
	Rats		
		Succumbing Within 1¾ to 5 hr.	
5	25	0	
10	16	7	
15	14	11	
20	13	8	
30	1	1	
40	5	5	

varying from 600 to 2000 units per kg. In adult rats (table 1), dosages from 5 to 40 U per kg. caused the animals to succumb within 1¾ to 5 hours. The prolonged survival period of the infant was not due to failure of the insulin to lower blood sugar. The average blood-sugar value of the infant rats was 91 mg. per cent before insulin was injected. One hour after injection the average value was 36 mg. per cent; 2 hours after, 28; 4 hours, 23; 6 hours, 26; and 8 hours after, 23 mg. per cent. These values for blood sugar are observed with the lowest dose of insulin adequate to produce profound hypoglycemia. Any increase of insulin dosage above that value up to 2000 U per kg. causes no change in the rate at which the blood sugar falls.

b) The toxic dose of insulin for adult cats and rats is approximately the same, 10 to 15 U per kg., but the cats succumbed to the acute effects of hypoglycemia in 4 to 16 hours and, therefore, lived longer than the rats (table 1). Some of the cats died after they had recovered from hypoglycemia.

TABLE 2. EFFECT OF HYPOGLYCEMIA AND HYPERGLYCEMIA ON SURVIVAL PERIOD OF ADULT RATS RESPIRING 4.5 TO 5 PER CENT OXYGEN IN NITROGEN

	Number of Rats	Average Time of Death in Minutes
Control	17	27
5 U per kg., insulin subcutaneously 1 hr. before exposure	16	15
5 U per kg., insulin subcutaneously 1 hr. before exposure and 2 gm./kg. glucose intravenously immediately before exposure	6	28
2 gm./kg., glucose intravenously immediately before exposure	8	26

c) The average survival period of adult male rats (weight 220 and 350 gm.) subjected to the inhalation of 4.5 to 5 per cent oxygen in nitrogen was 27 minutes (table 2). If these animals received an injection of insulin adequate to produce profound hypoglycemia, 5 U per kg., one hour before being subjected to anoxia the average survival period was shortened to 15 minutes. When animals, similarly treated with insulin, received an intravenous injection of glucose before exposure to nitrogen, the duration of the survival period in anoxia was restored to 28 minutes. Hyperglycemia produced by the injection of glucose, 2 gm. per kg. intravenously, into rats not injected with insulin caused no change in the duration of survival in anoxia.

Confirming previous observations on rats, it was found that 9-day-old kittens succumbed to anoxia more rapidly if they had received, an hour previously, 5 U per kg. of insulin. One control lived 96 minutes and two injected animals, 24 and 25 minutes, respectively, in 4.5 to 5 per cent oxygen.

TABLE 3. BLOOD SUGAR CONCENTRATION OF MALE RATS (150 TO 220 GM.) RESPIRING 4.5 TO 5 PER CENT OXYGEN IN NITROGEN FROM 5 TO 30 MINUTES

	Time minutes	Fed		Fasted	
		No. Observ.	Average Mg. %	No. Observ.	Average Mg. %
Controls		4	111	5	81
Oxygen in nitrogen					
4.5 to 5%	5	4	126	4	94
4.5 to 5%	10	4	150	5	54
4.5 to 5%	20	4	187	5	43
4.5 to 5%	30	5	228	4	29

d) It will be observed (table 3) that during anoxia the blood sugar rose in the fed, and fell in the fasted rats. In neither case, however, was there any effect on the duration of the survival period.

DISCUSSION

It will be easier to make a comparison of the results of hypoglycemia and anoxia in the infant rats, as well as to compare the differ-

ences in the reactions of the infants and adults to hypoglycemia and anoxia if the previous observations on infants are briefly summarized. *a*) In anoxia, infant rats live longer than adults. *b*) Hypoglycemia shortens the period of survival of anoxia in infant animals. *c*) Hyperglycemia prolongs the duration of the period of survival of anoxia. The three comparable results of the present investigation will be presented in the same order.

a) The greater ability of the infant than the adult rat to withstand hypoglycemia is in keeping with its increased resistance to anoxia. Since the blood sugar fell in infants as rapidly and deeply as in adults, it must be concluded that the infant survived longer because it is more resistant to hypoglycemia than is the adult. Probably the same factors as those which accounted for the more prolonged survival period of the newborn rat during anoxia (Himwich, *et al.*, 1942), namely, lower cerebral metabolic rate and a lower temperature of the entire body, including the brain of the poikilothermic infant, may also explain the resistance of the newborn rat to hypoglycemia. The capacity of the newborn to withstand hypoglycemia is not limited to rats. The human newborn, despite a low blood sugar, may not exhibit the clinical symptoms of hypoglycemia (Herrick and Tillman, 1938).

b) The combined effects of hypoglycemia and anoxia are similar in infant and adult rats for in a mixture of 4.5 to 5 per cent oxygen in nitrogen, hypoglycemia shortens the survival period of anoxia in adult rats, chiefly. The low level of blood sugar decreases the carbohydrate available for cerebral energy. The prime importance of the level of blood sugar is indicated by the fact that the injection of glucose into hypoglycemic animals restored the survival period of anoxia to its previous length.

c) In adult rats, rendered hyperglycemic by intravenous injections of glucose, the survival period of anoxia was not prolonged beyond the time of those not injected with glucose. The adult rat, therefore, differs from the infant in which the survival period of anoxia is prolonged by the injection of glucose. Since there was partial oxygen deprivation the prolongation of life must come from energy produced by glycolysis. Previous work has proved that the survival of anoxic infants depends upon their acquisition of anaerobic energy (Himwich, *et al.*, 1942; Tyler and Van Harreveld, 1942). The infant with lower brain metabolism is able to survive longer on this limited source of energy than the adult with its higher cerebral metabolic rate.

The previous nutritive condition of the animal had no influence on the duration of the survival period of the rat. That the higher level of blood sugar in the fed adults did not prolong the survival period is not surprising since the injection of 2 gm. of glucose per kg. did not influence the duration of the survival period either. In view of the fact that hypoglycemia decreases survival time, it might appear contradictory that our fasted rats did not succumb more rapidly than the fed ones

but it must be recalled that in the experiments in which hypoglycemia shortened the survival period the insulin was injected one hour before and the blood sugar had descended to hypoglycemic levels before the rats were subjected to anoxia. In our fasted rats the fall of blood sugar began to take place only after the anoxia was initiated.

It is interesting that cats live longer than the rats during hypoglycemia. This greater resistance may be associated with a lower rate of brain metabolism in the cat. Such a difference in brain metabolism between the two species was demonstrated by comparative respiratory studies on excised cerebral tissues (Quastel and Wheatley, 1932). On the other hand, in contrast with rats, some of the cats which did survive the hypoglycemia showed signs of irreversible cerebral damage. These included paralyses of various kinds. In the rat the cerebral hemispheres exhibit a higher rate of metabolism than that of any other part of the brain (Himwich, Sykowski and Fazekas, 1941; Tyler and Van Harreveld, 1942). It would, therefore, be the first part of the brain to be injured by hypoglycemia. But in the rat the motor centers are distributed over the dorsal surface of the hemispheres so that destruction of considerable portions of the cortex, including the motor fields, does not seriously interfere with the motor activity of these rats (Lashley, 1920–21). The metabolic rates of the various parts of the cat brain have not been studied. The cat is higher than the rat in the phyletic scale and has a greater degree of localization of function in the various portions of the cerebral cortex, hence destruction of any part of the brain, including the limited motor area, is more likely to leave a neurological residuum.

SUMMARY AND CONCLUSIONS

Infant rats survive hypoglycemia longer than adults. The factor determining the difference in the survival period is the ability to maintain the function of the brain during hypoglycemia. This capacity of the infant brain to endure hypoglycemia is associated with its lower cerebral metabolic requirements.

The survival period of the anoxic infant, but not of the anoxic adult, is prolonged by hyperglycemia. The adult cannot obtain sufficient energy from glycolysis to sustain the higher rate of cerebral metabolism. Hypoglycemia, however, shortens the survival period of anoxia for both infant and adult rats.

The fasted rat, in contrast with the fed one, suffers a fall in blood sugar while exposed to anoxia. The higher level of blood sugar in the fed adult rat, however, does not prolong the survival period of anoxia.

The dose of insulin producing death in rats and cats is approximately the same. The survival period of hypoglycemia is longer in the cat than in the rat. On the other hand the cat is more likely to exhibit neurological residua after recovery from hypoglycemia.

REFERENCES

FAZEKAS, J. F., F. A. D. ALEXANDER AND H. E. HIMWICH: *Am. J. Physiol.* 134: 281. 1941.
HAGEDORN, H. C., AND B. N. JENSEN: *Biochem. Ztschr.* 135: 46. 1923.
HERRICK, W. W., AND A. J. B. TILLMAN: *Surg. Gyn. & Obstet.* 66: 37. 1938.
HIMWICH, H. E., AND L. H. NAHUM: *Am. J. Physiol.* 101: 446. 1932.
HIMWICH, H. E., P. SYKOWSKI AND J. F. FAZEKAS: *Am. J. Physiol.* 132: 293. 1941.
HIMWICH, H. E., A. O. BERNSTEIN, H. HERRLICH, A. CHESLER AND J. F. FAZEKAS: *Am. J. Physiol.* 135: 387. 1942.
KABAT, H.: *Am. J. Physiol.* 130: 588. 1940.
LASHLEY, K. S.: *Brain* 43–44: 18. 1920–21.
QUASTEL, J. H., AND A. H. M. WHEATLEY: *Biochem. J.* 26: 725. 1932.
TYLER, D. B., AND A. VAN HARREVELD: *Am. J. Physiol.* 136: 600. 1942.

ANTAGONISM OF PITUITARY ADRENOCORTICOTROPIC HORMONE TO GROWTH HORMONE IN HYPOPHYSECTOMIZED RATS[1]

WALTER MARX, MIRIAM E. SIMPSON, CHOH HAO LI AND HERBERT M. EVANS

From the Institute of Experimental Biology, University of California
BERKELEY, CALIFORNIA

It was observed by Moon (1937) that pituitary extracts rich in adrenocorticotropic hormone inhibited somatic growth in young castrated rats. Extracts of the adrenal cortex and, in particular, purified adrenal cortical hormones such as corticosterone and related compounds which have an oxygen atom on carbon atom 11 of the perhydro-cyclopenteno-phenanthrene nucleus, have been found to have similar effects in normal rats (Ingle, Higgins and Kendall, 1938).

The mechanism of this growth inhibition is not understood. It may be mediated by the pituitary, or it may be due to direct metabolic effects of the adrenal cortical hormones. Since the hormones were administered at high dosage levels, the results may even have been due to non-specific or 'toxic' effects, resulting for example in decreased food consumption.

In the present communication, results are reported on the effect of adrenocorticotropic hormone on the action of the growth hormone in the absence of the pituitary.

EXPERIMENTAL PROCEDURE

Female rats were hypophysectomized at 26 to 28 days of age. In all experiments the animals were distributed in 4 groups; the first group was injected with growth hormone; the second group received the same dose of growth hormone, and simultaneously, adrenocorticotropic hormone; the third group was injected with the adrenocorticotropic hormone only, at the same dosage level as in the second group; the fourth group served as a control. Hormone administration was begun approximately 2 weeks after operation. The animals received intraperitoneal injections of the preparations daily for 15 days (13 injections),[2] and were killed 24 hours after the last injection.

As criteria for growth hormone activity *a*) body weights were measured at the beginning of the injection period and at 5-day intervals, and *b*) bone growth was determined by measuring the width of the uncalcified epiphyseal

Received for publication June 10, 1943.

[1] Aided by grants from the Board of Research of the University of California, from the Rockefeller Foundation, New York City, and from the National Research Council Committee on Research in Endocrinology.

[2] In some experiments, the doses of adrenocorticotropic hormone were distributed in two injections daily, since it was found that the activity of this hormone was markedly increased when its dosage was divided for more frequent administration (unpublished data).

cartilage of the tibiae stained with silver nitrate (Evans, Simpson, Marx and Kibrick, 1943).

The rats were given McCollum's 'diet I' in unrestricted amounts and the food consumption was measured.

Three different growth hormone preparations made from beef anterior pituitary were used. Two were cysteine-treated globulin fractions, and one was a preparation further purified by fractionation by varying the pH (Marx, Simpson and Evans, 1943). The preparations contained approximately 30, 50 and 100 growth hormone units per mg., respectively, (10-day test in hypophysectomized rats, Marx, Simpson and Evans, 1942). No gonadotropic, adrenocorticotropic or thyrotropic contaminations were demonstrable in these preparations when assayed in hypophysectomized rats at a total dose of 2.5 to 5.0 mg. (4-day test). Lactogenic hormone could not be demonstrated in such preparations when injected in total doses of 10.0 mg. into immature pigeons (4-day systemic test).

The adrenocorticotropic hormone, made from sheep pituitaries, was pure as demonstrated by biological tests and physico-chemical measurements (Li, Simpson and Evans, 1942).

RESULTS

The body weight determinations are summarized in table 1. Growth hormone alone produced weight gains of 15 to 26 gm. in 15 days. Simultaneous administration of adrenocorticotropic hormone counteracted almost completely the action of the growth hormone; the doubly-treated animals gained only 2 to 8 gm.

TABLE 1. EFFECTS OF PITUITARY GROWTH (GH) AND ADRENOCORTICOTROPIC (ACTH) HORMONES ON BODY WEIGHT IN HYPOPHYSECTOMIZED RATS

Daily Dosage Levels		Number of Rats	Average Body Weight Increase in 15 Days			
GH	ACTH		GH alone	GH+ACTH	ACTH alone	Controls
mg.	*mg.*		*gm.*	*gm.*	*gm.*	*gm.*
0.08	3.0	33	26	8	3	3
0.08	2.0	21	19	2	−7	−4
0.09	1.0	30	15	5	−6	−2
0.10	0.1	24	16	6	−4	−4

The effects of these hormones alone and combined on the width of the uncalcified portion of the proximal epiphyseal cartilage of the tibia are summarized in table 2. These measurements indicated that the effects on epiphyseal growth were similar to those on body growth. Growth hormone caused a marked increase in the width of the cartilaginous disc. This effect of growth hormone was almost completely nullified when adrenocorticotropic hormone was injected simultaneously with growth hormone; adrenocorticotropic hormone administered alone caused no increase, possibly even a decrease, in the width of the epiphysis.

The determinations of the daily food consumption are summarized in table 3 and the results indicate that the amount was similar in the

TABLE 2. EFFECTS OF PITUITARY GROWTH (GH) AND ADRENOCORTICOTROPIC (ACTH) HORMONES ON THE UNCALCIFIED PORTION OF THE PROXIMAL EPIPHYSEAL CARTILAGE OF THE TIBIA IN HYPOPHYSECTOMIZED RATS

Daily Dosage Levels		Number of Rats	Width of Cartilage			
GH	ACTH		GH alone	GH+ACTH	ACTH alone	Controls
mg.	*mg.*		*mm.*	*mm.*	*mm.*	*mm.*
0.08	3.0	23	0.36	0.17	0.12	0.14
0.09	1.0	30	0.35	0.17	0.08	0.15
0.10	0.1	24	0.30	0.20	0.13	0.16

three groups of injected rats. It is evident that rats treated simultaneously with growth hormone and adrenocorticotropic hormone did not eat less, but if anything, slightly more than the animals receiving the same dose of growth hormone alone. The food consumption of the controls was smaller than that of any experimental group.

TABLE 3. EFFECTS OF PITUITARY GROWTH (GH) AND ADRENOCORTICOTROPIC (ACTH) HORMONES ON THE FOOD INTAKE OF HYPOPHYSECTOMIZED RATS

Daily Dosage Levels		Average Daily Food Intake Per Rat[1]			
GH	ACTH	GH alone	GH+ACTH	ACTH alone	Controls
mg.	*mg.*	*gm.*	*gm.*	*gm.*	*gm.*
0.08	3.0	5.2	6.2	5.9	4.6
0.08	2.0	4.8	4.9	4.5	2.3
0.09	1.0	3.5	5.2	4.5	3.6

[1] Average values from a total of 10 determinations on 94 rats.

In each experiment autopsies were performed at the termination of the injection period in order to ascertain the completeness of hypophysectomy. Adrenal and thymus weights were also determined in order to observe the effects of the two hormones on these organs. The results are summarized in table 4. The adrenocorticotropic hormone caused, as anticipated, a marked increase in adrenal weights. In the groups treated with growth hormone alone, the adrenals were slightly heavier than in the control groups.[3] In 3 of 4 experiments the combination of the two hormones also produced slightly heavier adrenal glands than adrenocorticotropic hormone alone. In the case of the thymus the two hormones caused, as anticipated, opposite effects (Moon, 1937; Uyldert and Freud, 1938). The high dosage levels of adrenocorticotropic hormone reduced thymus weights to approximately a fourth of those of the control animals, whereas thymus weights of rats given growth hormone were approximately twice those of controls. In the groups receiving both hormones, the adrenocorticotropic hormone had the dominating influence, causing almost the same degree of thymic involution as the adrenocorticotropic hormone alone.

[3] These slightly heavier adrenals did not show the characteristic histological changes resulting from minimal doses of adrenocorticotropic hormone.

TABLE 4. EFFECT OF PITUITARY GROWTH (GH) AND ADRENOCORTICOTROPIC (ACTH) HORMONES ON WEIGHTS OF ADRENALS AND THYMUS IN HYPOPHYSECTOMIZED RATS

Daily Dosage Levels		Number of Rats	Average Weights of Adrenals and Thymus, mg.							
			GH alone		GH+ACTH		ACTH alone		Controls	
GH	ACTH		Adr.	Thym.	Adr.	Thym.	Adr.	Thym.	Adr.	Thym.
mg.	*mg.*									
0.08	3.0	34	13	223	27	30	22	26	9	119
0.08	2.0	30	16	250	29	27	27	24	12	104
0.09	1.0	30	12	222	35	27	23	27	10	125
0.10	0.1	24	14	199	19	71	21	109	12	134

DISCUSSION

One might assume that the observed phenomena are related to specific metabolic effects of the two hormones. It has been demonstrated that growth hormone causes formation of tissue protein (Lee, 1938), positive nitrogen balance (Teel and Cushing, 1930) and depression of liver arginase (Fraenkel-Conrat, Simpson and Evans, 1943). Hormones of the adrenal cortex, on the other hand cause a negative nitrogen balance (Long, Katzin and Fry, 1940), and activation of liver arginase (Fraenkel-Conrat, *et al.*, 1943). These considerations, indicating that certain metabolic responses are influenced in an opposite direction by the two hormones, must be borne in mind in the interpretation of the antagonism of adrenocorticotropic hormone to growth hormone.

SUMMARY

The action of pituitary growth hormone, as demonstrated both by body weight increase and by proliferation of the proximal epiphyseal cartilage of the tibia, is inhibited by simultaneous administration of pituitary adrenocorticotropic hormone. This antagonism occurs in the absence of the pituitary gland. It can not be attributed to a reduction in the food consumption. It would appear to result from opposing metabolic effects of these two hormones.

REFERENCES

EVANS, H. M., M. E. SIMPSON, W. MARX AND E. KIBRICK: *Endocrinology* 32: 13. 1943.
FRAENKEL-CONRAT, F., M. E. SIMPSON AND H. M. EVANS: *Am. J. Physiol.* 138: 439. 1943.
INGLE, D. J., S. M. HIGGINS AND E. C. KENDALL: *Anat. Rec.* 71: 363. 1938.
LEE, M. O.: *Assoc. Research Nerv. & Mental Dis.* 17: 193. 1938.
LI, C. H., M. E. SIMPSON AND H. M. EVANS: *Science* 96: 450. 1942.
LONG, C. N. H., B. KATZIN AND E. G. FRY: *Endocrinology* 26: 309. 1940.
MARX, W., M. E. SIMPSON AND H. M. EVANS: *Endocrinology* 30: 1. 1942.
MARX, W., M. E. SIMPSON AND H. M. EVANS: *J. Biol. Chem.* 147: 77. 1943.
MOON, H. D.: *Proc. Soc. Exper. Biol. & Med.* 37: 34. 1937.
TEEL, H. M., AND H. CUSHING: *Endocrinology* 14: 157. 1930.
UYLDERT, T. E., AND J. FREUD: *Acta brev. Neerland.* 8: 188. 1938.

INCREASED SODIUM CHLORIDE AND WATER INTAKE OF NORMAL RATS TREATED WITH DESOXYCORTICOSTERONE ACETATE[1]

KATHERINE K. RICE AND CURT P. RICHTER

From the Psychobiological Laboratory, Phipps Psychiatric Clinic, The Johns Hopkins Hospital

BALTIMORE, MARYLAND

In previous self-selection experiments it was found that adrenalectomized rats showed a greatly increased appetite for sodium chloride and that when given access to salt separately from their food they took enough to keep themselves alive and free from symptoms of insufficiency (Richter, 1936). It was further found that after implantation of adrenal tissue or after treatment with 1-desoxycorticosterone acetate the adrenalectomized rats reduced their salt intake to normal amounts (Richter and Eckert, 1938). Thus by their appetites the untreated adrenalectomized rats indicated that they needed salt, while those in which the adrenal cortical insufficiency was corrected indicated that they did not need additional salt.

We have now applied this method of appetite study to the investigation of the changes in sodium chloride and water needs produced by injections of desoxycorticosterone acetate in normal animals. We were interested to determine whether such rats would indicate by their appetite that they needed less or more sodium chloride. In view of several recent reports that normal animals treated with desoxycorticosterone acetate manifest a diabetes insipidus-like syndrome, we were also interested to determine whether, when given full opportunity to regulate their sodium chloride needs, such animals would still show an increased thirst (Ragan, *et al.*, 1940; Harrop and Thorn, 1937; Thorn, *et al.*, 1938; 1941; Clinton and Thorn, 1942; Mulinos, *et al.*, 1941; Silvette and Britton, 1938; Kuhlmann, *et al.*, 1939; Corey, *et al.*, 1939).

METHODS

The experiments to be reported were carried out in 3 series. In the first series 8 adult male rats were kept in individual cages and given free access to a 3 per cent solution of sodium chloride, a 1 per cent solution of potassium chloride, tap water, and our stock diet made without added salt and with unsalted butter.[2] Records were made daily of the food and fluid intake, and weekly of the body weight. Fluids were changed twice weekly. After 15 days

Received for publication June 16, 1943.

[1] Aided by a grant from the Committee for Research in Endocrinology of the National Research Council.

[2] Graham flour, 725; crude casein (Labco), 100; skim milk powder (Breadlac), 100; saltless butter, 50; calcium carbonate, 15.

during which normal values were obtained, the rats were given daily subcutaneous injections of desoxycorticosterone acetate[3] in increasing dosage. In the second series 4 adult male rats, also in individual cages, at first were given access only to the salt-poor stock food and tap water; after the average intake levels had been determined, they were given daily subcutaneous injections of 2.5 mg. of desoxycorticosterone acetate. Twenty days later the tap water was replaced by a 1 per cent solution of sodium chloride and the

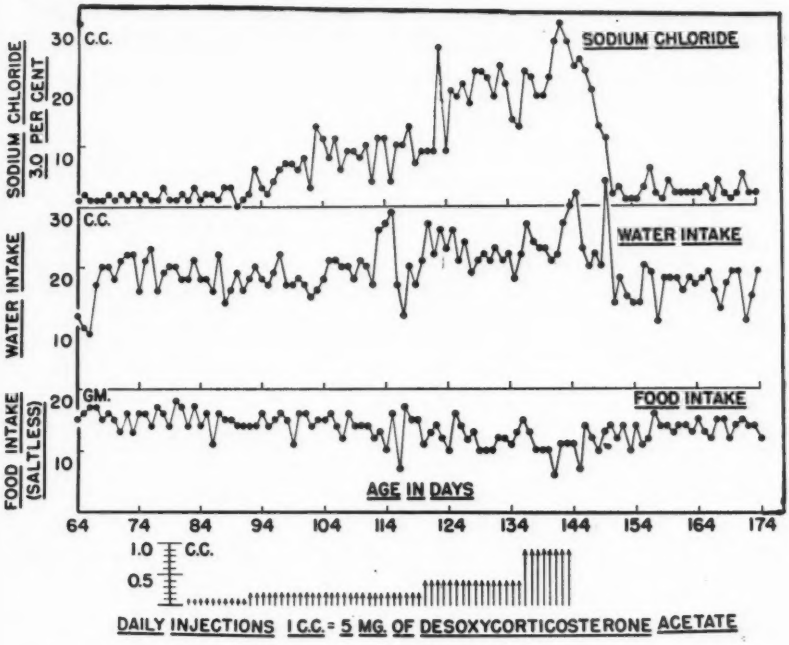

Fig. 1. Typical record showing the effects produced by daily injections of desoxycorticosterone acetate on the intake of a 3 per cent solution of sodium chloride, water and food.

daily injections of desoxycorticosterone acetate continued. Data were again recorded as above. In the third series 2 groups of 4 adult male rats each were given access, in individual cages, respectively to the low-salt stock food and tap water, and to a high-salt food (5 per cent sodium chloride added to the regular food) and tap water. After 16 days daily injections of 2.5 mg. of desoxycorticosterone acetate were begun, and their influence on the food and water intake and body weight recorded.

RESULTS

First series. Figure 1 gives a record of the food, sodium chloride and water intake of one of the 8 rats in the first group. This rat was

[3] The desoxycorticosterone acetate (Percorten) used for these experiments was supplied through the courtesy of Ciba Pharmaceutical Products, Inc., Summit, N. J., and contained 5 mg. of 1-desoxycorticosterone acetate per cc. of sesame oil.

placed in the experimental cage at an age of 64 days, observed for a period of 15 days, and then given daily injections for 62 days of desoxycorticosterone acetate in increasing concentrations (0.5 mg. for the first 10 days, 1 mg. for the next 29 days, 2 mg. for the following 15 days, and 4 mg. for the last 8 days), after which it was observed for an additional 31 days.

As can be seen in the figure, the injection of doses of 0.5 mg. of desoxycorticosterone acetate had no significant effect on the intake of sodium chloride, water or food. However, on the day following the

TABLE 1. INFLUENCE OF DESOXYCORTICOSTERONE ACETATE (DCA) ON THE INTAKE OF 3 PER CENT SODIUM CHLORIDE, TOTAL FLUID AND SALT-POOR FOOD

Series I	3% Sodium Chloride, cc.			Total Fluid, cc.			Salt-Poor Food, gm.		
Rat No.	Last 10 days before DCA	Last 10 days with DCA	11–20th days after DCA	Last 10 days before DCA	Last 10 days with DCA	11–20th days after DCA	Last 10 days before DCA	Last 10 days with DCA	11–20th days after DCA
323Y	1.5	20.4	2.3	23.9	46.1	20.9	15.7	11.0	13.5
324Y	3.8	13.8	1.1	26.3	39.9	21.8	14.9	10.1	13.9
325Y	2.3	37.9	4.6	34.4	88.9	26.7	16.1	12.4	13.4
326Y	5.4	19.6	1.1	27.5	46.1	19.6	16.2	10.8	13.8
327Y	1.6	13.0	0.7	23.0	44.5	18.0	15.3	9.5	12.6
328Y	2.4	18.5	1.1	21.7	54.4	19.6	14.7	12.0	12.8
329Y	2.7	16.9	1.1	18.7	43.0	14.9	15.1	10.6	12.6
330Y	1.9	16.4	1.3	21.7	45.9	17.1	14.9	10.9	12.5
Average	2.7	19.6	1.7	24.7	51.1	19.8	15.4	10.9	13.1

doubling of the dose the intake of sodium chloride was more than doubled and after 10 days had increased more than four-fold. Further doubling of the dose, to 2 mg. of hormone per day, again more than doubled the intake of the salt solution, but a dose of 4 mg. per day produced only a slight further increase in salt appetite. The potassium chloride intake was not appreciably affected by the treatment, varying between 2.3 and 5.0 cc. of the 1 per cent solution per day, independently of the hormone dosage. The water intake, however, was somewhat increased, so that with the increase in sodium chloride, the total fluid intake of the rat was approximately twice as great with the larger hormone dosages as it had been pre-experimentally. The daily food ingestion of the animal showed a slow, steady decrease from an average of 15.9 gm. during the last 10 days before injections to 8.6 gm. during the 8 days in which the largest doses, 4 mg. per day, of desoxycorticosterone acetate were being injected.

Table 1 summarizes the results of the observations made on the 8 rats in this group. The average daily sodium chloride intake increased from 2.7 cc. for the last 10 days before the start of the injec-

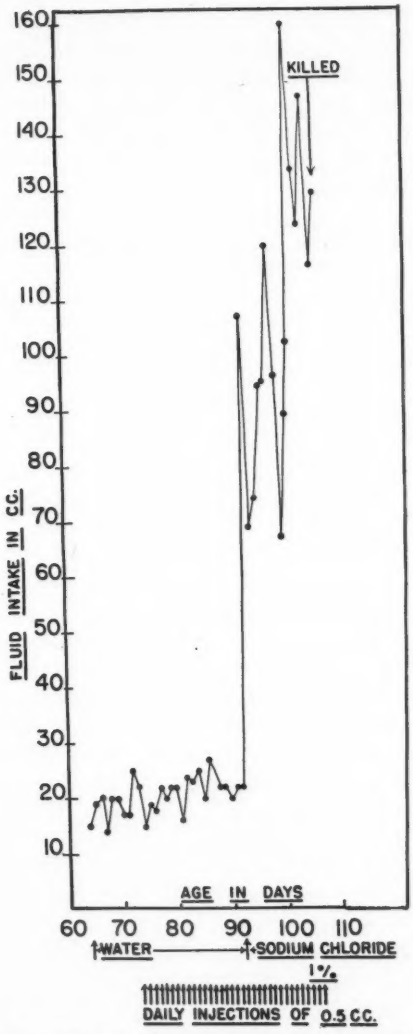

Fig. 2. Typical record showing the effects produced by daily injections of desoxycorticosterone acetate on the intake of water and of a 1 per cent solution of sodium chloride.

tions to 19.6 cc. for the last 10 days of the injection period, and decreased to 1.7 cc. for the 11 to 20th days following the last injection. The changes in salt intake were very similar in all 8 rats. The varia-

tions in potassium chloride intake were in most cases not large, but were sufficiently inconsistent that we have not felt able to interpret them without further experimentation. For this reason the data are not included at this time, except in the total fluid intake of the rats. The average daily total fluid intake increased from 24.7 cc. for the last 10 days before the injections were begun to 51.1 cc. for the last 10 days of the injection period, and decreased to 19.8 cc. for the following 11 to 20th day period. Here again all of the rats showed essentially the same changes. The average daily food intake decreased from 15.4 to 10.9 gm. and increased again to 13.1 gm. during the three periods, respectively.

TABLE 2. INFLUENCE OF DESOXYCORTICOSTERONE ACETATE (DCA) ON THE INTAKE OF FLUID WHEN 1% SODIUM CHLORIDE WAS SUBSTITUTED FOR WATER

Series 2 Rat No.	Cc. Water, Last 10 Days Before DCA	Cc. Water, 11–20th Days With DCA	Cc.% Salt Solution, 5–14th Days With DCA
324Y	23.4	29.4	78.3
325Y	17.8	22.7	117.5
326Y	16.9	22.8	119.0
327Y	16.2	18.2	30.5
Average	18.6	23.3	86.3

The results of these experiments showed that injections of desoxycorticosterone acetate definitely increased the appetite for salt and also increased the thirst of the animals. In nearly all instances the water intake closely paralleled the intake of sodium chloride.

Second series. The second series of experiments was then undertaken, to determine if desoxycorticosterone acetate acts directly to increase both the salt and the water intake, or whether it acts directly on the salt intake, and only indirectly on the water intake. In these experiments 4 rats were given daily injections of 2.5 mg. of desoxycorticosterone acetate, first during a period when they had access only to tap water and salt-poor stock food, and then during a period when the tap water was replaced by a 1 per cent solution of sodium chloride. Figure 2 shows the record of one of the 4 rats in this group. During the 10 days before the injections were begun the daily water intake averaged 17.8 cc., and during the period of 11 to 20 days after injections were started it averaged 22.7 cc. On the 21st day of the injections the tap water was replaced by a 1 per cent salt solution. On the following day the rat drank 107 cc., and in the period from 5 to 14 days on this régime the salt intake averaged 117.5 cc. per day. Table 2 summarizes the results of the observations on the 4 rats. From an average of 18.6 cc. during the last 10 days before injection, the daily intake of water increased to only 23.3 cc. during the last

10 days of injections of desoxycorticosterone acetate with access to plain water, while during the last 10 days of injections with access to 1 per cent sodium chloride solution the fluid intake averaged 86.3 cc.

Third series. The results of the previous experiments disproved a primary polydipsia as a result of desoxycorticosterone acetate injections in normal animals, since there was no appreciable increase in fluid intake until the water was replaced by 1 per cent salt solution.

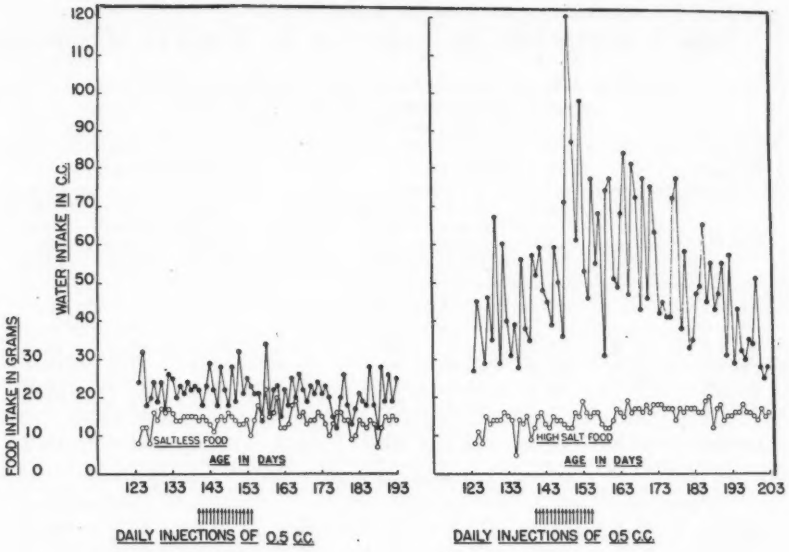

Fig. 3. Typical records showing the effects produced by daily injections of desoxycorticosterone acetate on the water intake of *a*) a rat kept on a low salt diet and *b*) a rat kept on a high salt diet.

On the other hand, they did not rule out the possibility of a secondary polydipsia as a consequence of the increased salt intake induced by the drug, since it was not clear whether the large volume of salt solution was taken for its salt content or also in order to satisfy the thirst produced by the increased salt consumption. In order to test this point the third series of experiments was made. Two groups of 4 rats each were used. The first group was given access to tap water and the basic low-salt food. The second group had access to tap water and a high-salt food made by adding 5 per cent of sodium chloride to the regular food.[4] After 17 days, during which base lines of food and water intake were obtained, the animals were given daily subcutaneous injections of 2.5 mg. of desoxycorticosterone acetate. Figure 3 shows

[4] The regular food is made with salted butter and contains 1.5% added sodium chloride.

the food and water intake of one rat in each group. The record of the animal on saltless food showed practically no change as a result of the treatment, with an average water intake of 22.5 cc. and 23.4 cc. for the 10 days before and the last 10 days of injections, respectively. The food intake was also unaffected. On the other hand, the rat on high-salt food increased its daily water intake from an average of 42.3 cc. during the last 10 days before, to 64 cc. during the last 10 days of treatment, thus indicating that the increased salt retention produced a secondary increase in water intake.

Table 3 summarizes the results for the 2 groups of animals.

TABLE 3. INTAKE OF WATER AND FOOD IN RATS TREATED WITH DESOXYCORTICOSTERONE ACETATE (DCA) ON A LOW-SALT AND HIGH-SALT FOOD

Series 3	Water Intake, cc.				Food Intake, gm.			
Rat No.	Last 10 days before DCA	Last 10 days with DCA	First 10 days after DCA	31–40th days after DCA	Last 10 days before DCA	Last 10 days with DCA	First 10 days after DCA	31–40th days after DCA
Low-salt food								
223AA	22.9	22.8	19.9	22.7	14.8	10.9	15.0	13.9
224AA	22.5	23.4	20.6	21.1	15.4	13.8	15.7	13.1
225AA	20.5	16.5	15.5	21.3	13.9	11.2	13.4	13.6
226AA	25.3	22.0	24.4	23.6	13.9	10.5	15.8	12.4
Average	22.8	21.2	20.1	22.2	14.5	11.5	14.9	13.3
High-salt food								
227AA	46.0	72.9	85.8	61.2	12.6	16.0	16.7	15.5
228AA	42.3	64.0	61.8	49.4	12.9	14.8	16.7	15.8
229AA	35.6	59.9	54.1	42.8	13.7	16.2	16.0	14.4
230AA	45.9	55.6	56.3	45.4	15.7	14.5	17.1	15.6
Average	42.4	63.1	64.5	49.9	13.7	15.4	16.6	15.3

Whereas the water intake of the 4 rats on the low-salt diet was practically unaffected by the desoxycorticosterone acetate, that of the animals on the high-salt food showed a pronounced effect. From a daily average of 42.4 cc., before injections were begun, which was already nearly double the water consumption of the low-salt group, the intake increased to 63.1 cc. during the injection period. Moreover, 40 days after the injections were stopped the daily water intake was still 49.9 cc., *i.e.*, 20 per cent higher than the pre-experimental level. The food intake of the two groups of rats was also interesting. The animals on the low-salt diet showed a small but consistent decrease in food consumption during treatment, from an average of 14.5 to 11.5 gm., with a prompt return to normal on the cessation of injections. The rats on the high-salt food, on the other hand, increased their food intake from 13.7 to 15.4 gm., presumably because of the induced salt

craving, during treatment, and maintained this increase until the experiments were terminated 40 days after the last injections.

DISCUSSION

The results of biochemical studies have shown that the injection of desoxycorticosterone produces a salt retention in both normal and adrenalectomized animals (Harrop and Thorn, 1937; Thorn, Engel and Eisenberg, 1938; Regan, *et al.*, 1940). In keeping with this observation results of self-selection studies have shown that when treated with desoxycorticosterone, adrenalectomized rats lost their greatly increased appetite for salt, thus no longer indicating a need for increased amounts of salt (Richter and Eckert, 1938). That in normal rats desoxycorticosterone should produce just the opposite effect, an increased salt appetite, was an unexpected result. By their appetite these normal, treated animals have also indicated an increased salt need during treatment.

In view of our observations on adrenalectomized rats the possibility had to be considered in the first place that in normal rats desoxycorticosterone may produce an increased salt excretion rather than a retention. Thorn and others (Thorn, Engel and Lewis, 1941; Clinton and Thorn, 1942) have shown that some of the cortical hormones do have this effect, but that the opposite holds true for desoxycorticosterone. The second possibility may then be considered that although desoxycorticosterone produces a salt retention, the salt either is withdrawn from the blood to the tissues, or is held in the blood in an unavailable form. The increased calcium appetite of parathyroidectomized rats may be considered, at least in part, as an instance of an increased appetite for a mineral which is retained in the body, but not readily available for purposes of metabolism. The excess of cortical material, that is, the natural secretion from the adrenal glands in addition to the injected desoxycorticosterone, as well as the effect of the desoxycorticosterone on the activity of the gland, must be considered as additional factors which may have contributed to the end result, the increased salt appetite.

Our results would indicate further that the increased salt appetite is primary and that the increased water intake is secondary, since the rats showed an increased water intake only when salt was being retained in the body. Ragan and his co-workers (1940) have also produced polydipsia and polyuria in normal rats on a high salt diet by the administration of desoxycorticosterone, but were unable to produce the same effects in rats on a low-sodium and low-potassium diet. Mulinos and his associates (1941) similarly concluded from their experiments on injected dogs, "it appears that the magnitude of the excessive fluid exchange which results from small doses of desoxycorticosterone acetate is dependent on the amount of sodium chloride in the diet."

However, most of the workers in this field have interpreted the increased fluid intake of normal animals injected with cortical hormone as a manifestation of a diabetes insipidus-like syndrome, rather than as a direct consequence of the induced sodium retention (Mulinos, *et al.*, 1941; Silvette and Britton, 1938; Kuhlmann, *et al.*, 1939; Corey, *et al.*, 1939; Selye and Basset, 1940; Corey and Britton, 1941; Ferrebee, *et al.*, 1941; Moehlig and Jaffe, 1942). Thus, in the same paper in which Mulinos (1941) points out the close relationship between the abnormal thirst of the treated animals and the salt content of their diet, he postulates that the 'diabetes insipidus' produced is the result of an imbalance between the anti-diuretic activity of the posterior pituitary gland and the diuretic activity of the adrenal cortex. Similarly Silvette and Britton (1938) discuss the antagonistic effect of cortico-adrenal and posterior pituitary hormones on the kidney. On the other hand Ragan (1940) has pointed out that the polydipsia and polyuria produced by desoxycorticosterone acetate injections, do not respond significantly to the administration of pitressin. Our own experiments would suggest that the posterior pituitary hormone is not necessarily involved, at least directly, in the adjustment of the animal to the increased desoxycorticosterone concentration. The increased water intake is so clearly a direct consequence of the sodium chloride retention that it can be interpreted as a necessary measure for the maintenance of proper osmotic pressure relationships in the animal.

SUMMARY

Eight normal male rats, given free access to a salt-poor diet, a 3 per cent solution of sodium chloride, a 1 per cent solution of potassium chloride, and tap water, showed an approximately 7-fold increase in appetite for the sodium solution when given daily injections of from 2 to 4 mg. of desoxycorticosterone acetate.

Four rats on a salt-poor diet and tap water showed no increased thirst when treated with the adrenal cortical hormone, but showed a more than 3-fold increase in fluid intake when the tap water was replaced by a 1 per cent solution of sodium chloride.

Four rats given access to a high-salt food (5% added sodium chloride) and tap water showed approximately a 50 per cent increase in water intake when given daily injections of 2.5 mg. of desoxycorticosterone acetate, while 4 control rats on a salt-poor diet and tap water showed no increase in thirst.

On the basis of these data it is suggested that the primary effect of desoxycorticosterone administration in normal rats is an increased salt need, resulting in an increased salt appetite.

It is further suggested that the polydipsia of animals treated with desoxycorticosterone acetate on a high-salt intake is a direct consequence of the latter rather than a manifestation of a primary effect on water metabolism.

REFERENCES

CLINTON, M., JR., AND G. W. THORN: *Science* 96: 343. 1942.
COREY, E. L., AND S. W. BRITTON: *Am. J. Physiol.* 133; 511. 1941.
COREY, E. L., H. SILVETTE AND S. W. BRITTON: *Am. J. Physiol.* 125: 644. 1939.
FERREBEE, J. W., D. PARKER, W. H. CARNES, M. K. GERITY, D. W. ATCHLEY AND R. F. LOEB: *Am. J. Physiol.* 135: 230. 1941.
HARROP, G. A., AND G. W. THORN: *J. Exper. Med.* 65: 757. 1937.
KUHLMANN, D., C. RAGAN, J. W. FERREBEE, D. W. ATCHLEY AND R. F. LOEB: *Science* 90: 496. 1939.
MOEHLIG, R. C., AND L. JAFFE: *J. Lab. & Clin. Med.* 27: 1009. 1942.
MULINOS, M. G., C. L. SPINGARN AND M. E. LOJKIN: *Am. J. Physiol.* 135: 102. 1941.
RAGAN, C., J. W. FERREBEE, P. PHYFE, D. W. ATCHLEY AND R. F. LOEB: *Am. J. Physiol.* 131: 73. 1940.
RICHTER, C. P.: *Am. J. Physiol.* 115: 155. 1936.
RICHTER, C. P., AND J. F. ECKERT: *Endocrinology* 22: 214. 1938.
SELYE, H., AND L. BASSET: *Proc. Soc. Exper. Biol. & Med.* 45: 272. 1940.
SILVETTE, H., AND S. W. BRITTON: *Science* 88: 150. 1938.
THORN, G. W., L. L. ENGEL AND H. EISENBERG: *J. Exper. Med.* 68: 161. 1938.
THORN, G. W., L. L. ENGEL AND R. A. LEWIS: *Science* 94: 348. 1941.

NOTES AND COMMENTS

PREPARATION OF AN ACTIVE FRACTION OF THE PINEAL GLAND

In a former publication (Fischer, 1938), a method of biological assay of pineal extracts was described and the isolation of an active fraction of the gland was reported. The results of the biological assay were confirmed by Engel (1939).

Since the method of preparation of the active pineal extract was given only in general terms by Engel, it is made now in a complete and detailed form.

The biological assay was based upon the facts, that: a) In 90 per cent of infantile female mice, 22 days of age and weighing 8 gm., the intact vaginal membrane opened within 6 days. b) An active fraction of the pineal gland delayed the time of opening of the vaginal membrane. As 3 μg. of this preparation was necessary to inhibit this opening within the 6-day period, this quantity was adopted as an arbitrary unit. The inhibition period was prolonged for 10 days by using 25 μg. Higher doses were only a little more effective (inhibition for 11 days by 100 μg.). The inhibition test was regarded as positive when of 6 treated animals no more than one, and of 6 control animals at least 5 showed an open membrane. In most cases none of the treated animals and all of the control animals had open vaginas.

The active fraction was prepared in the following manner: Five hundred gm. of frozen pineal glands were ground and extracted consecutively with 10 liters of 95 per cent alcohol, 5 liters of acetone and 5 liters of ether. The extracted mass was dried at room temperature and pulverized.

Seventy-five gm. of dry powder was obtained which was then stirred for 2 hours with a mixture of 720 cc. of distilled water and 30 cc. of 25 per cent NH_4OH. This procedure was repeated with a fresh mixture of the solvent after centrifuging. The two clear extracts were combined and the pH adjusted to 4.7 (colorimetrically). At this point a precipitate appeared which was filtered off. The clear solution was then saturated with picric acid at room temperature. The precipitate was filtered off (this precipitate contains a substance similar in its effects to Evans' synergic factor) and washed with a saturated solution of picric acid. The solutions after being combined, were precipitated with a ten-fold amount of acetone, containing 10 per cent of ether. The following day the precipitate was centrifuged, washed several times with acetone to eliminate completely the picric acid and finally washed with ether and dried at room temperature. The dry substance was dissolved in 100 cc. of 50 per cent alcohol and adjusted to pH 8.5. After filtering, the pH of the extract was adjusted to 6.5 (colorimetrically), at which a precipitate appeared, which was filtered off. (It should be determined whether an-

Received for publication June 7, 1943.

other precipitate can be obtained by varying the pH, which should be eliminated. In most of our experiments the only precipitate was obtained at pH 6.5.) The clear solution was precipitated with a mixture of 1 liter of dried acetone and 100 cc. of dried ether. The precipitate was washed with acetone and ether and finally dried at room temperature. Yield: 1.65 gm. of white powder.

As stated above, an arbitrary unit is contained in 3 μg. of this powder. The activity was destroyed by heating a solution of 0.005 per cent (5 μg. in 0.1 cc.) to 100°C. for 30 minutes.

Summary. A detailed description of the preparation of a pineal gland fraction is given, which showed activity in the biological assay.

ENGEL, P: *Endocrinology* 25: 144. 1939.
FISCHER, E.: *Arch. Internat. Pharmacodynamie* 49: 340. 1938.

E. FISCHER

From the Department for Chemical Investigations, Instituto Bacteriologico de Chile, Santiago, Chile

NEW BOOKS

CHEMISTRY AND METHODS OF ENZYMES. James B. Sumner, Professor of Biochemistry, Cornell University and G. Fred Somers, Instructor in Biochemistry, Cornell University, New York City. Cloth. $5.00. xii and 365; 6 illustrations; 9 figures; 26 tables. New York City, Academic Press. 1943.

THIS book contains a brief summary and short discussion of the pertinent facts about most of the known enzymes. The first part of the book (44 pages) is devoted to a discussion of the properties of enzymes in general, and the final chapter (34 pages) contains a review of the reactions involved in carbohydrate metabolism "as an example of how enzymic reactions may work together to carry out a complicated biological process." The remainder of the book is composed of discussions of individual enzymes, and each enzyme is considered from the standpoints of history, occurrence, action, specificity, activity measurement, activation and inactivation, preparation and properties. The methods of measuring the activity of each enzyme, as well as the procedures used in the preparation of crystalline enzymes, are usually given in some detail. The hydrolytic enzymes include esterases, carbohydrases, enzymes of carbohydrate metabolism, nucleases, amidases and proteolytic enzymes. The oxidases are subdivided into those containing iron or copper, and the dehydrogenases are classified as 1) containing riboflavin, 2) containing coenzyme I or II, and 3) enzymes that pass hydrogen to cytochrome. A miscellaneous group of oxidizing enzymes and a number of desmolases, hydrases, and mutases are also discussed.

The book was written "to give the research worker and advanced student a general survey of enzyme chemistry without presenting too much detail on any one subject." This has been done very well, and its greatest value lies in the fact that it is one of the few books that presents a comprehensive survey of the entire enzyme field in a very readable manner. It will be of particular value to the beginnner who is desirous of obtaining a bird's-eye view of the entire enzyme field, and it will also have a place as a handy reference book in which most of the important facts about an enzyme can be found readily. The brief discussions of individual enzymes will not fulfill the needs of a research worker or advanced student of enzyme chemistry.

W. W. WESTERFELD

FACTS FOR CHILDLESS COUPLES. E. C. Hamblen, M.D. Associate professor of Obstetrics and Gynecology, Duke University School of Medicine; Chief of the Endocrine Division of the Department of Obstetrics and Gynecology and Endocrinologist, Duke Hospital, Durham, North Carolina. 103 pages; 11 text figures. Cloth binding. Springfield, Illinois, Charles C Thomas, Publisher. 1942.

THIS handbook is so detailed and technical that it might well serve as a guide for a physician in the investigation of sterility. For the exceptional couple who wish to understand the minutiae of medical procedure it will furnish a wealth of information. One wonders, however, if such specific instructions are wise. Although routines for sterility investigations are similar in their broad outlines, they vary widely in particulars from clinic to clinic; and for the average patient the technical details cited in this book might lead to lack of confidence in the physician and poor co-operation.

G. E. SEEGAR JONES

ASSOCIATION NOTICE

ANNOUNCEMENT of winners in the Schering Award Competition of 1942 has been made by the Committee of Judges. The subject in the second competition of the Schering Award was "Endocrinology in War Medicine" or certain approved alternate subjects. The winners are: *1st prize:* One full year's scholarship to Elizabeth L. Brown, Class of 1943, New York Medical College, "Endocrines in the Nervous System." *2nd prize:* One-half year's scholarship to Eugene B. Brody, Class of 1944, Harvard Medical School, "Hormone Factors in Personality." *3rd prize:* $100.00 to Roslyn Wiener, Class of 1945, University of Michigan Medical School, "Role of Hormones in Pregnancy and Parturition."

The Schering Award Competition is offered annually by the Schering Corporation for the purpose of stimulating a current interest in endocrinology among undergraduate medical students. It is sponsored and administered by a special committee of the Association of Internes and Medical Students and the competition is now in its third year. The following students have warranted honorable mention, and through the courtesy of the Schering Corporation, they will receive a one-year subscription to either ENDOCRINOLOGY or the JOURNAL OF CLINICAL ENDOCRINOLOGY: N. G. Demy, Marquette University; N. Josephson, Yale Medical School; S. Kafka, Middlesex University; G. V. Mann, Johns-Hopkins; L. W. Pratt, Johns-Hopkins; A. Segaloff, Wayne University; A. J. Swyer, University of Vermont; G. Turtletaub, Middelsex University; E. W. Amyes, College of Medical Evangelists; C. Cohen, Loyola University; and A. P. Rosen, Long Island College of Medicine.

The Committee of Judges in the Schering Award of 1942 comprised eight outstanding American investigators in the fields of endocrinology, medicine and chemistry: R. G. Hoskins, Director of Memorial Foundation for Neuro-Endocrine Research, Harvard Medical School, and Editor of the Journal of Clinical Endocrinology; E. P. McCullagh, Section of Endocrinology and Metabolism, the Cleveland Clinic; E. C. Hamblen, Associate Professor and Chief of the Endocrine Division, Department of Obstetrics and Gynecology, Duke University School of Medicine; E. Novak, Associate Professor of Obstetrics, University of Maryland School of Medicine and College of Physicians and Surgeons; H. M. Evans, Institute of Experimental Biology, University of California; F. C. Koch, Chairman of the Department of Biochemistry, University of Chicago; E. L. Sevringhaus, Professor of Medicine, University of Wisconsin Medical School; and E. Shorr, Assistant Professor of Medicine, Cornell University Medical College, and the New York Hospital.

At the present time, plans for the Schering Award Competition of 1943 are being formulated by the Association of Internes and Medical Students in conjunction with the Schering Corporation. Particulars of subject matter and other details will be announced shortly.